Application
for
United States Letters Patent

To all whom it may concern:

Be it known that Ronald Breslow et al.

have invented certain new and useful improvements in

BETA-CYCLODEXTRIN DIMERS AND PHTHALOCYANINES AND USES THEREOF

of which the following is a full, clear and exact description.

Dkt. 57474-A/JPW/ADM

BETA-CYCLODEXTRIN DIMERS AND PHTHALOCYANINES AND USES THEREOF

The invention disclosed herein was made with Government support under grant No. GM-18754 from the National Institutes of Health, U.S. Department of Health and Human Services, and CHE-97-12556 from the National Science Foundation. Accordingly, the U.S. Government has certain rights in this invention.

Background Of The Invention

Throughout this application, various publications are referenced in parentheses. Full citations for these references may be found at the end of the specification immediately preceding the claims. The disclosures of these publications in their entireties are hereby incorporated by reference into this application to more fully describe the state of the art to which this invention pertains.

Photodynamic therapy of cancers uses a combination of light-activated drugs (photosensitizers) and laser light to create highly reactive forms of oxygen (singlet oxygen) that destroy tumor cells (Ali et al. 1999, Dougherty et al. 1998, Sternberg et al. 1998). Porphyrinoid dyes are photosensitizers which are widely used in photodynamic therapy. However, one major drawback of these hydrophobic photosensitizers is that they are not selective to tumor tissue because they are transported to every organ by blood lipoproteins of the blood stream (Moser et al., 1994). One way to prevent this is to attach the

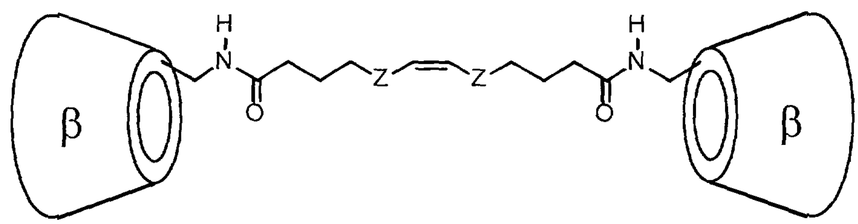
photosensitizer to cancer-specific antibodies and use cyclodextrin dimers to encapsulate the dye so that it cannot interact with lipoproteins (Ruebner et al., 1996, 1997).

Another strategy involves the use of β-cyclodextrin dimers having a cleavable linker between two β-cyclodextrin molecules to deliver the photosensitizer to the tumor site (U.S. Serial No. 09/352,529, filed July 13, 1999, now allowed; Ruebner et al. 1999). The β-cyclodextrin dimers can serve as hydrophilic carriers for photosensitizers, which can be administered to a subject with cancer. The β-cyclodextrin dimer can be cleaved by light, which can be selectively directed at the tumor site. The dye will then be released and be able to go into tumor cells. After the dimer is cleaved, the concentration of uncleaved β-cyclodextrin dimers at the tumor site will be reduced and more uncleaved β-cyclodextrin dimers will diffuse into the tumor site due to the concentration gradient. In this way, photosensitizer can be concentrated in the tumor without the use of a cancer-specific antibody.

The phthalocyanine described in U.S. Serial No. 09/352,529, filed July 13, 1999, now allowed, and in Ruebner et al. 1999 was a mixture of eight compounds. The present application discloses phthalocyanines with single well-defined structures, additional β-cyclodextrin dimers that can be used in photodynamic therapy, and phthalocyanines having characteristics that permit efficient cleavage of the β-cyclodextrin dimer-phthalocyanine complex.

Summary Of The Invention

The invention provides a composition of matter comprising two β-cyclodextrin molecules and a cleavable linker joining each such β-cyclodextrin, wherein the cleavable linker comprises a carbon-carbon double bond substituted on both ends, wherein the cleavable linker is cleavable by singlet oxygen, and wherein the composition of matter is selected from the group consisting of:



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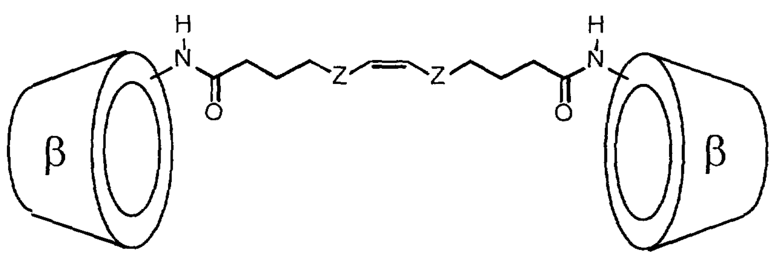

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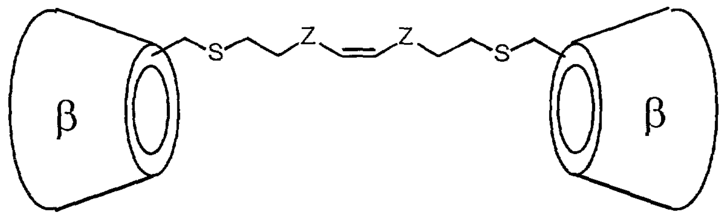

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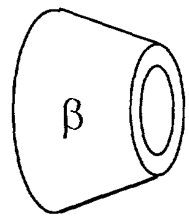
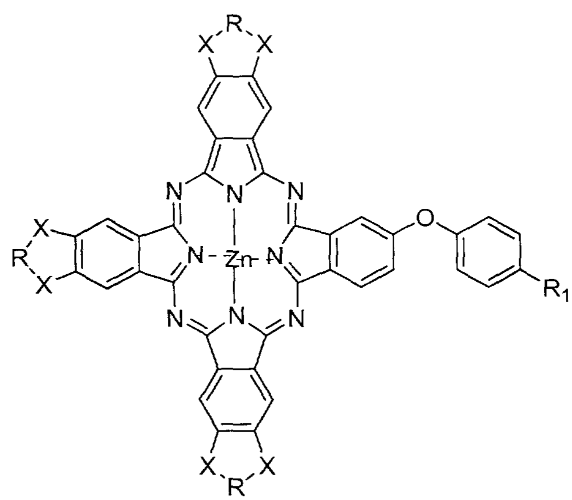


= beta-cyclodextrin; and

wherein Z is C_1-C_4 alkyl, NH, N(C_1-C_4 alkyl), O, or S.

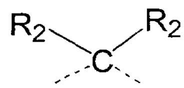
The invention also provides a compound having the structure:



wherein X is C_1-C_4 alkyl, NH, N(C_1-C_4 alkyl), O, or S;

wherein R_1 is $-CO_2H$, $-CO_2^-$, $-N^+(CH_3)_3$, $-SO_3H$, or $-SO_3^-$; and

wherein R is



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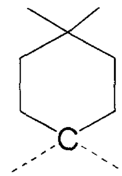

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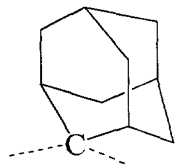

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where the dashed lines indicate the attachments to X, and where R_2 is C_1-C_3 alkyl.

Brief Description Of The Figures

FIGURE 1. β-Cyclodextrin dimers with photocleavable linkers as carriers for photosensitizers in the photodynamic therapy of cancers. The linker joining the β-cyclodextrin molecules **1** is cleavable by photoirradiation causing the release of the photosensitizer, which in the case illustrated is phthalocyanine **2**.

FIGURE 2. Synthesis of β-cyclodextrin dimers **17** and **18**. Detailed description of synthesis is in text.

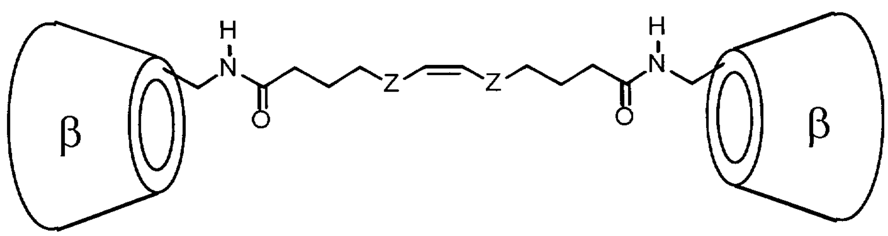
FIGURE 3. Synthesis of β-cyclodextrin dimer **21**. Detailed description of synthesis is in text.

FIGURE 4. Determination of binding constant for β-cyclodextrin dimer **21** with BNS and with phthalocyanine **13**. -x-: BNS titration into dimer **21** solution; -o-: BNS titration into phthalocyanine **13**:dimer **21** (1:1) solution.

FIGURE 5a-5e. Conversion of β-cyclodextrin dimer **21** to its cleavage product upon irradiation, with phthalocyanine **11**, monitored by NMR. Reaction time: a, 30 minutes; b, 60 minutes; c, 90 minutes; d, 120 minutes; e, 150 minutes. Chemical shifts reported in parts per million (ppm) downfield of zero on the delta (δ) scale (x-axis).

Detailed Description Of The Invention

The invention provides a composition of matter comprising two β-cyclodextrin molecules and a cleavable linker joining each such β-cyclodextrin, wherein the cleavable linker comprises a carbon-carbon double bond substituted on both ends, wherein the cleavable linker is cleavable by singlet oxygen, and wherein the composition of matter is selected from the group consisting of:



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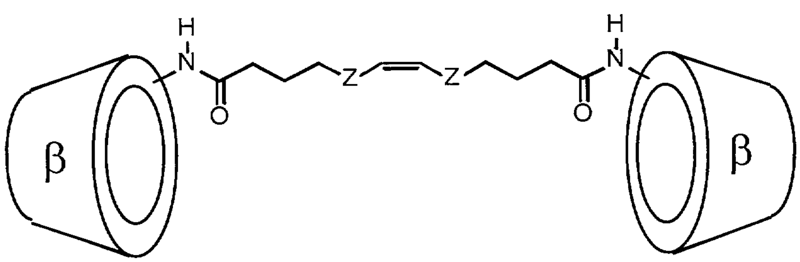

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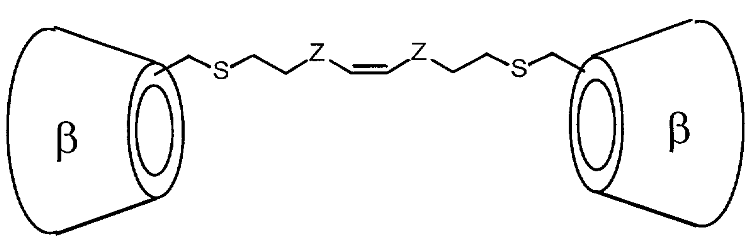

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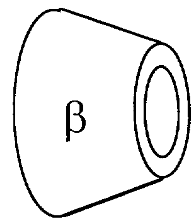
wherein

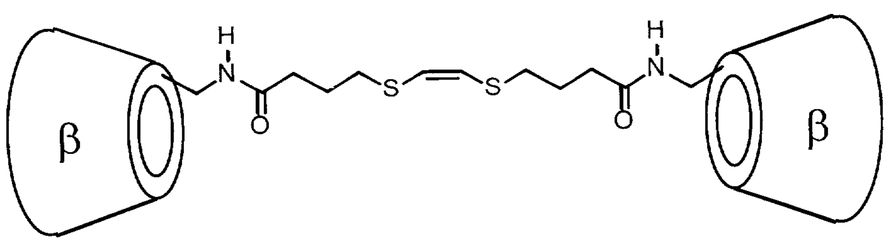


= beta-cyclodextrin; and

wherein Z is C_1-C_4 alkyl, NH, N(C_1-C_4 alkyl), O, or S.

In one embodiment of the composition of matter, Z is S.

In one embodiment, the composition of matter has the structure:



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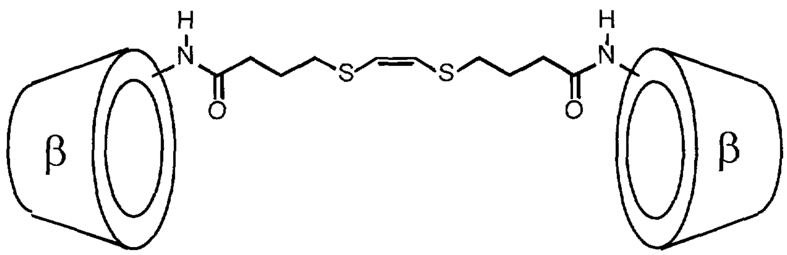
In one embodiment, the composition of matter has the structure:



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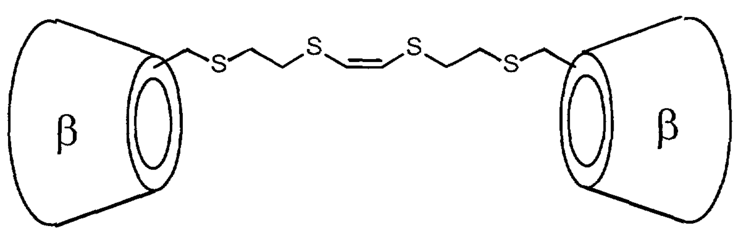
In one embodiment, the composition of matter has the structure:



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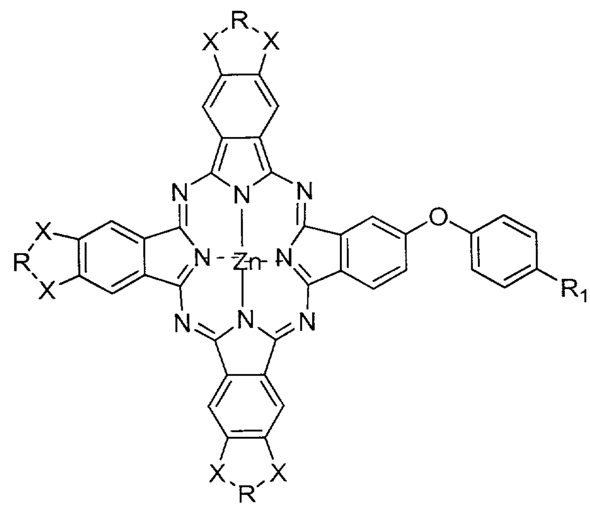
β-Cyclodextrin dimers with linkers of different lengths can be used to accommodate different size photosensitizers.

The invention provides a composition which comprises a hydrophilic matrix comprising the any of the compositions of matter disclosed herein and a photosensitizer encapsulated within the matrix. In different embodiments, the photosensitizer is a porphyrin, a phthalocyanine, a naphthalocyanine, a chlorin, a pheophorbide, or a bacteriopheophorbide.

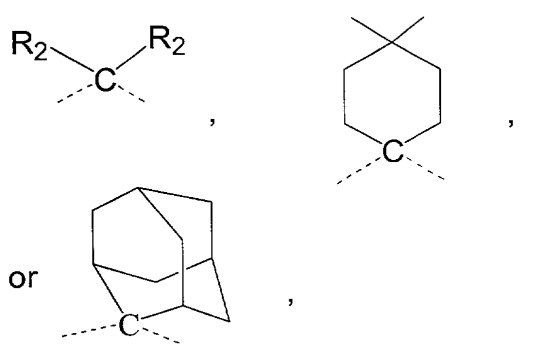
In one embodiment of the composition, the photosensitizer is a phthalocyanine. In one embodiment, the phthalocyanine has the structure:



wherein X is C_1-C_4 alkyl, NH, N(C_1-C_4 alkyl), O, or S;

wherein R_1 is $-CO_2H$, $-CO_2^-$, $-N^+(CH_3)_3$, $-SO_3H$, or $-SO_3^-$; and

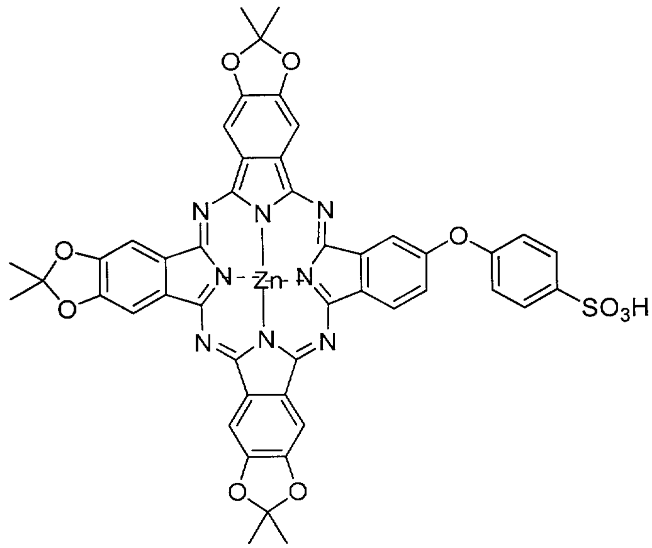
wherein R is



where the dashed lines indicate the attachments to X, and where R_2 is C_1-C_3 alkyl.

In one embodiment, X is O, and R_1 is $-SO_3H$.

In one embodiment, the phthalocyanine has the structure:



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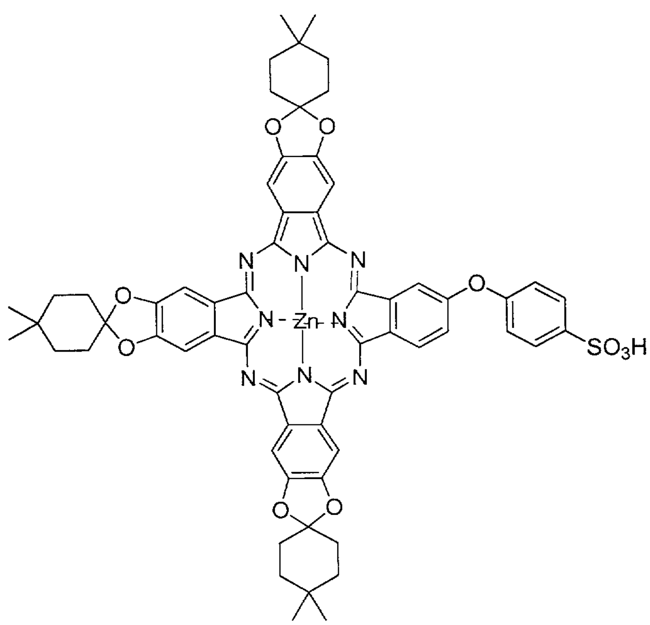
In one embodiment, the phthalocyanine has the structure:



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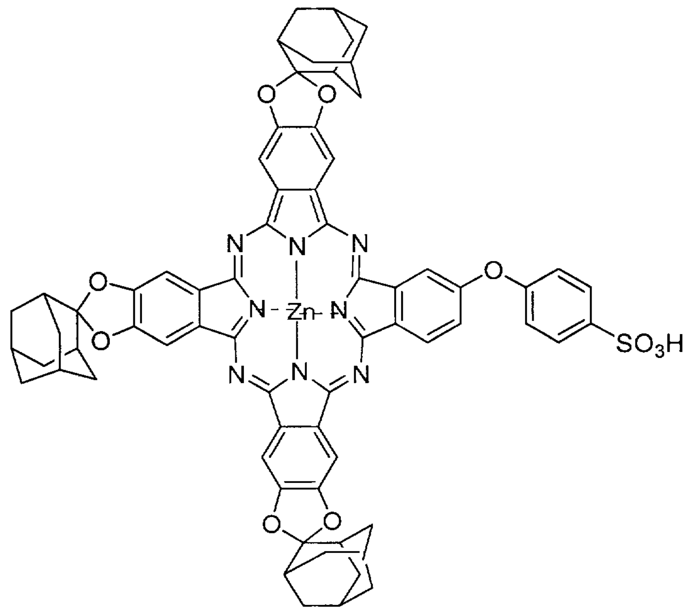
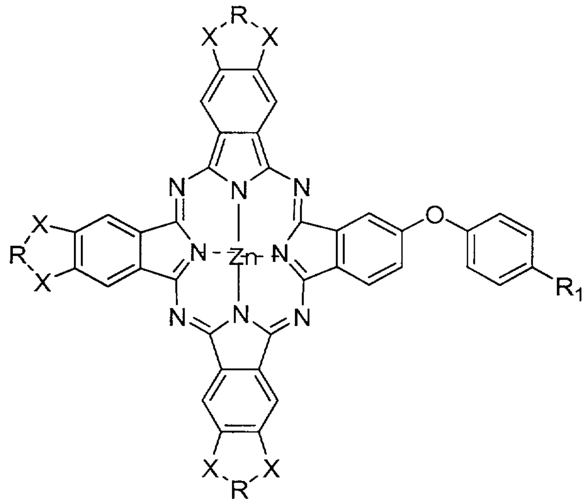
In one embodiment, the phthalocyanine has the structure:



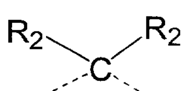
The invention provides a compound having the structure:



wherein X is C_1-C_4 alkyl, NH, N(C_1-C_4 alkyl), O, or S;

wherein R_1 is $-CO_2H$, $-CO_2^-$, $-N^+(CH_3)_3$, $-SO_3H$, or $-SO_3^-$; and

wherein R is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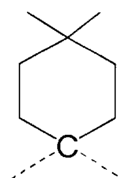
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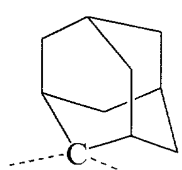
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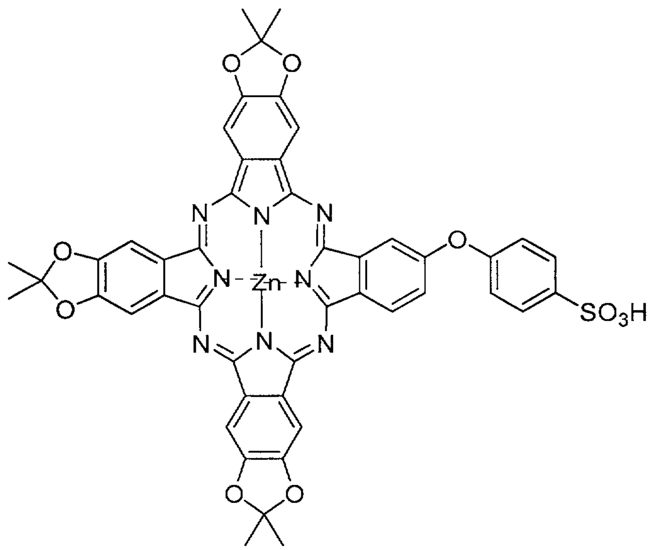
where the dashed lines indicate the attachments to X, and where R_2 is C_1-C_3 alkyl.

In one embodiment of the compound, X is O, and R_1 is $-SO_3H$.

In one embodiment, the compound has the structure:



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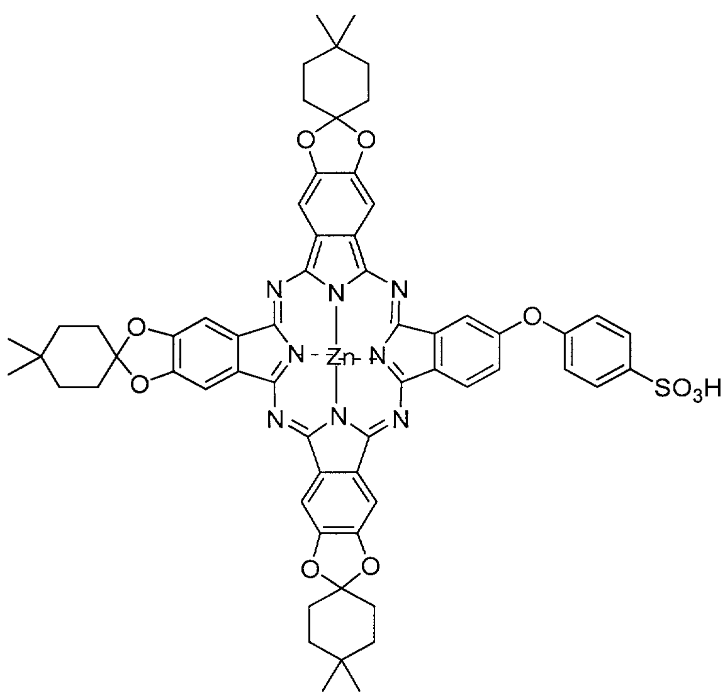
In one embodiment, the compound has the structure:



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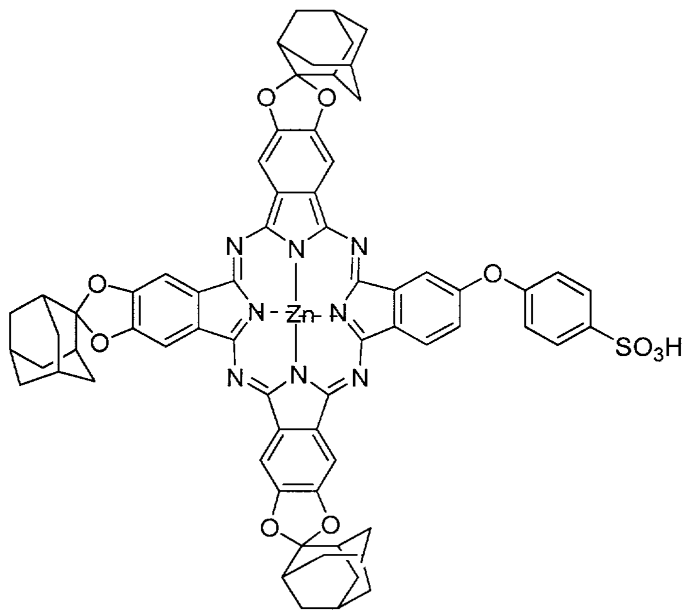
In one embodiment, the compound has the structure:



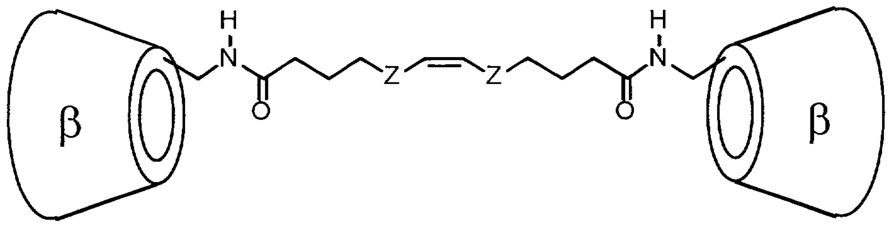
The invention provides a composition which comprises a hydrophilic matrix comprising:

i) any of the compounds disclosed herein encapsulated within the matrix, and

ii) a composition of matter comprising two β-cyclodextrin molecules and a cleavable linker joining each such β-cyclodextrin, wherein the cleavable linker comprises a carbon-carbon double bond substituted on one or both ends by an electron rich atom, and the cleavable linker is cleavable by singlet oxygen.

In different embodiments of the composition, the electron rich atom is sulfur, oxygen, or nitrogen.

In one embodiment of the composition, the composition of matter is selected from the group consisting of:



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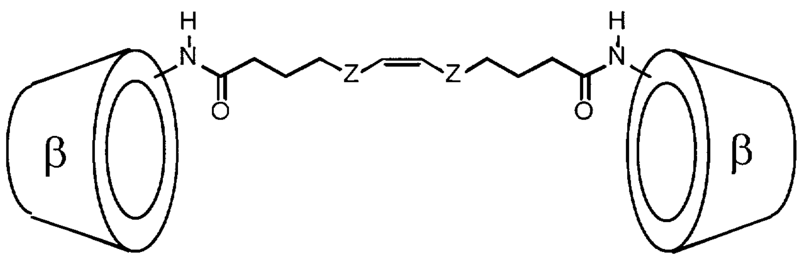

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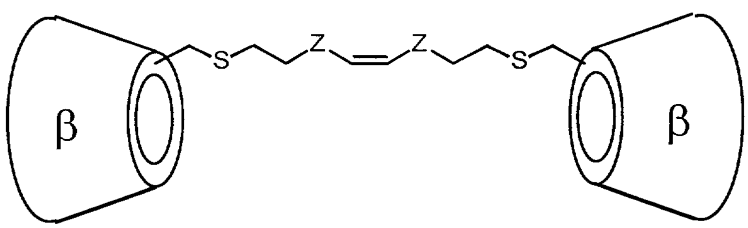

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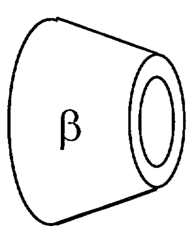
wherein

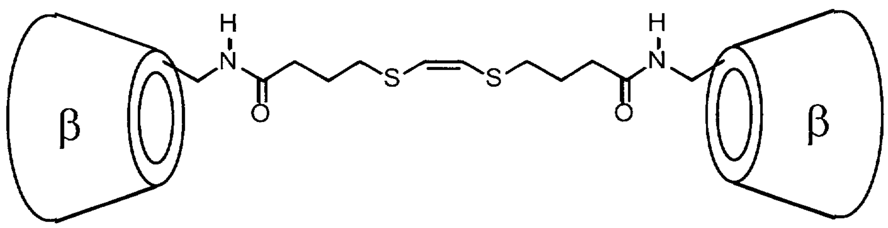


= beta-cyclodextrin; and

wherein Z is C_1-C_4 alkyl, NH, N(C_1-C_4 alkyl), O, or S.

In one embodiment, Z is S.

In one embodiment of the composition, the composition of matter has the structure:



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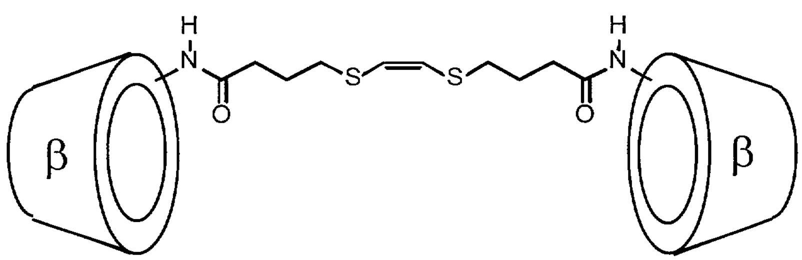
In one embodiment of the composition, the composition of matter has the structure:



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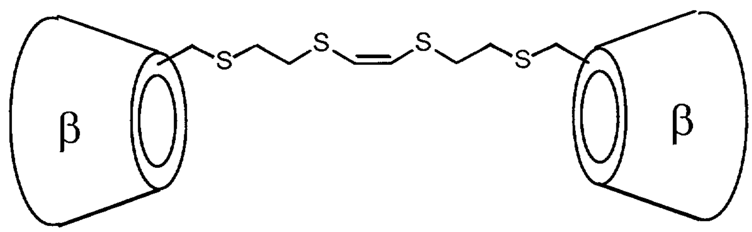
In one embodiment of the composition, the composition of matter has the structure:



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In one embodiment of any of the compositions disclosed herein, the cleavable linker is cleavable upon exposure to light of a wavelength appropriate for absorption by the photosensitizer. In one embodiment, the photosensitizer is released when the cleavable linker is cleaved.

The invention provides a method of killing a tumor cell which comprises contacting the tumor cell with any of the compositions disclosed herein and exposing the composition to light so as to cleave the cleavable linker and release the photosensitizer, wherein absorption of light by the photosensitizer excites the photosensitizer and the tumor cell is killed by singlet oxygen that is formed by energy transfer from the excited photosensitizer.

The invention provides a method of killing a tumor cell in a subject which comprises:

(a) administering any of the compositions disclosed herein to the subject;

(b) directing light at the tumor cell so as to expose the composition to light and cleave

the cleavable linker thereby releasing the photosensitizer, wherein absorption of light by the photosensitizer excites the photosensitizer and generates singlet oxygen that is formed by energy transfer from the excited photosensitizer;

(c) allowing additional composition to diffuse to the tumor cell; and

(d) repeating steps (b) and (c) until sufficient singlet oxygen is generated to kill the tumor cell.

In one embodiment of any of the methods disclosed herein, the photosensitizer is concentrated at the tumor cell.

In one embodiment of any of the methods disclosed herein, a plurality of converging light beams is used to focus light on the tumor cell.

The compositions of matter and compounds disclosed herein may also be useful in applications other than the photodynamic therapy of cancer.

This invention will be better understood from the Experimental Details which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims which follow thereafter.

Experimental Details

The following Experimental Details are set forth to aid in an understanding of the invention, and are not intended, and should not be construed, to limit in any way the invention set forth in the claims which follow thereafter.

Background

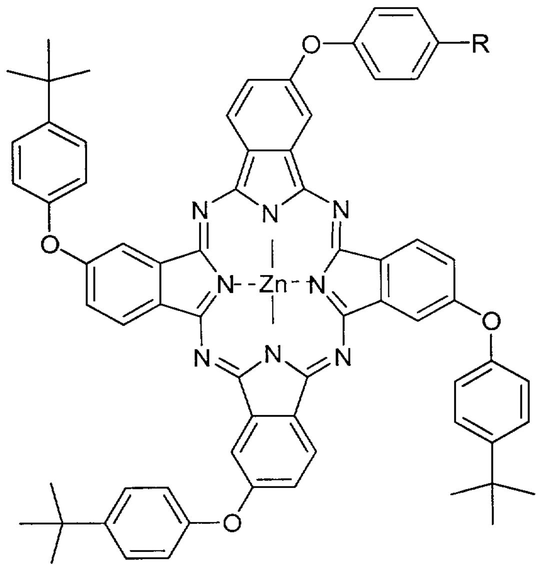
As previously disclosed (U.S. Serial No. 09/352,529, filed July 13, 1999, now allowed; Ruebner et al. 1999) and illustrated in **Figure 1**, β-cyclodextrin dimer **1** and zinc phthalocyanine **2** formed a complex that is soluble in water. On irradiation of the complex in the presence of oxygen, the double bond of **1** is cleaved by singlet oxygen to form two moles of thioformate **4**. Singlet oxygen adds to double bonds to form dioxetanes, which spontaneously fragment to generate carbonyl groups (Adam and Cliento 1983, Bartlett 1976, Clennan and Nagraba 1988, Foote 1971, Frimer 1979, Kearns 1971, Schaap and Zaklika 1979). The addition is particularly favorable for double bonds with electron donor substituents, as in **1**. Since dimeric binding is stronger than the monomeric binding that occurs once the linker is cleaved, **4** then dissociates from **2**. Furthermore, the chain of **4** almost certainly lowers the affinity of the cyclodextrin for the phthalocyanine by tucking back into the cyclodextrin cavity. An analog of **4** with a methyl group in place of the formyl group had an order of magnitude lower affinity for 4-*tert*-butylbenzoic acid than does simple β-cyclodextrin.

Phthalocyanine **2** is in fact a mixture of eight compounds, in which the substituents may be attached 3333 to the four phthalocyanine benzene rings (this is the structure shown, with the first number assigned as position 3 for the position of the sulfonate substituent on **2**), 3233, 3323, 3332, 3223, 3322, 3232, or 3222 (Marcuccio et al. 1985). As described below, the present application discloses phthalocyanines with single well-defined structures. Phthalocyanines were chosen over porphyrins, because phthalocyanines are more stable to oxidation.

Synthesis of zinc phthalocyanines

An overview of the synthesis procedures is described in this section. Details are described in the "Detailed Synthesis" section at the end of "Experimental Details".

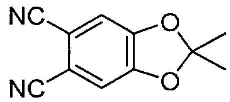
Using a standard phthalocyanine synthetic procedure (Leznoff 1989), then incorporation of zinc, compounds **5** and **6** were prepared. Compound **5** is an analog of **2** with four equivalent substituents (it is a mixture of only four isomers since the 3333 isomer is the same as the 2222 isomer). Compound **6** is also an analog of **2** with a carboxyl instead of a sulfonate solubilizing group (again a mixture of eight isomers) (Kliesch et al. 1995).



R = *tert*-butyl **5**
R = CO_2H **6**

To achieve the symmetry that would give a single isomeric zinc phthalocyanine, phthalonitrile **7** was synthesized. This was made by brominating the catechol acetonide (Mitchell and Lai, 1979), then displacing the bromines with CuCN (the Rosenmund-von Braun reaction) (reviewed in Ellis and Romney-Alexander 1987). Conversion to the phthalocyanine with Li in pentanol, then treatment with zinc acetate, afforded compound **8**. The elongated hydrophobically substituted **9** was synthesized from the corresponding phthalonitrile, synthesized by converting 3,4-dibromocatechol (Kohn 1951) to its

ketal using 4,4-dimethylcyclohexanone (Meyer et al 1985), then displacing with CuCN. This was converted to the fully symmetrical zinc phthalocyanine **9** in a one-pot procedure with Li and zinc acetate (for an example of such transformation, see Lawrence and Whitten 1996).



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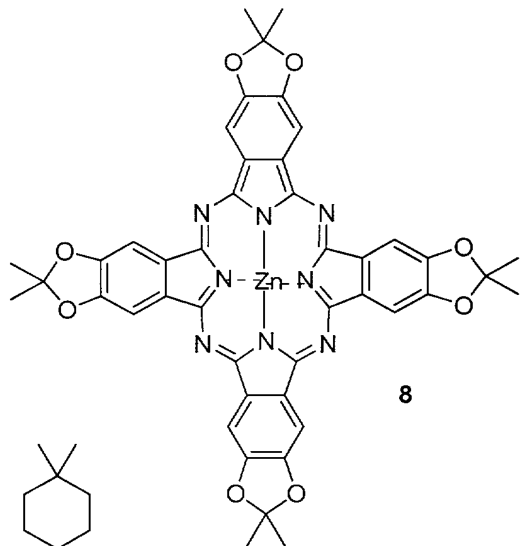

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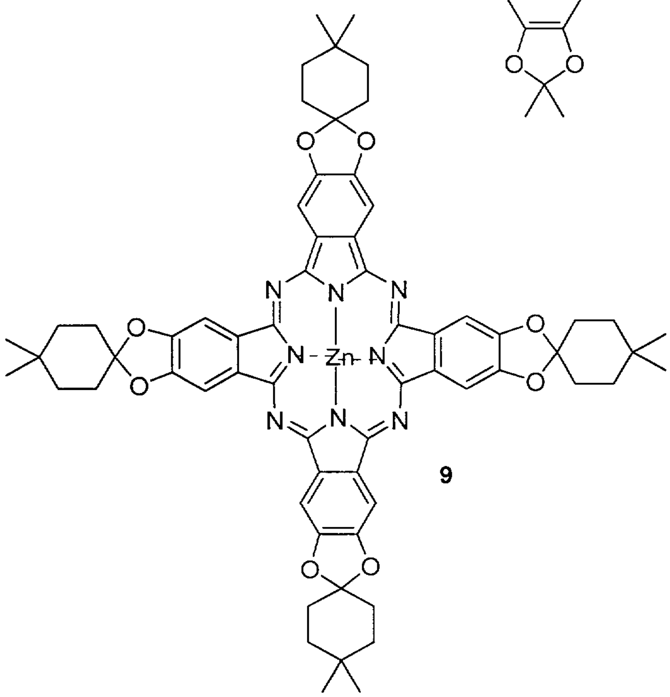

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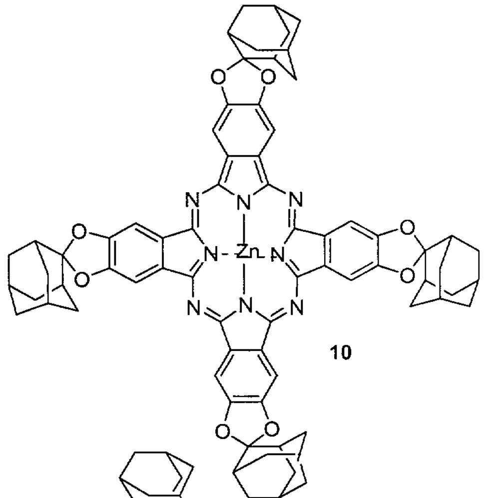
Adamantane derivatives bind well to β-cyclodextrin in water (Rekharsky and Inoue 1998), so zinc phthalocyanine **10** was also prepared from the ketal of catechol and 2-adamantanone (Takakis et al. 1992) by the bromination (Metz et al. 1984) and cyanide displacement sequence described herein, with the one-pot Li and Zn^{2+} method of cyclization. Two additional compounds were also prepared with one carboxyl solubilizing group, **11** and **12**, by using a mixture of phthalonitriles and then separating the products. In the same manner the three monosulfonated analogs **13**-**15** were prepared. These compounds are all single isomers.

Finally, the commercially available zinc phthalocyanine **16** (Aldrich Chemical Company, Milwaukee), which is smaller than the other phthalocyanines, was also used in the studies described below.



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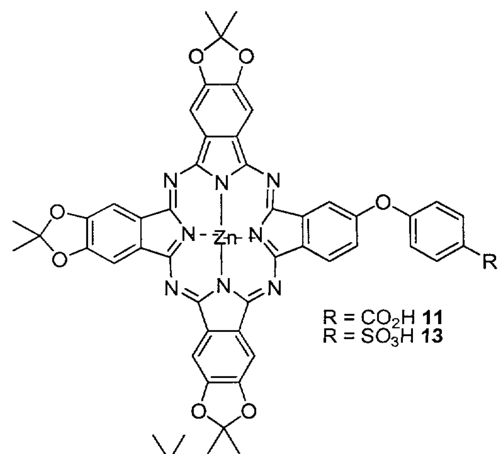
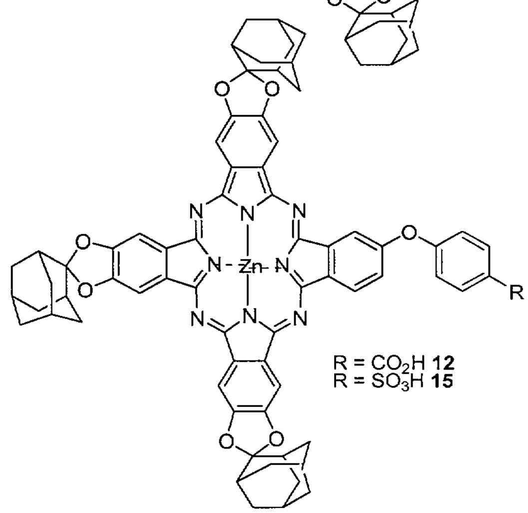
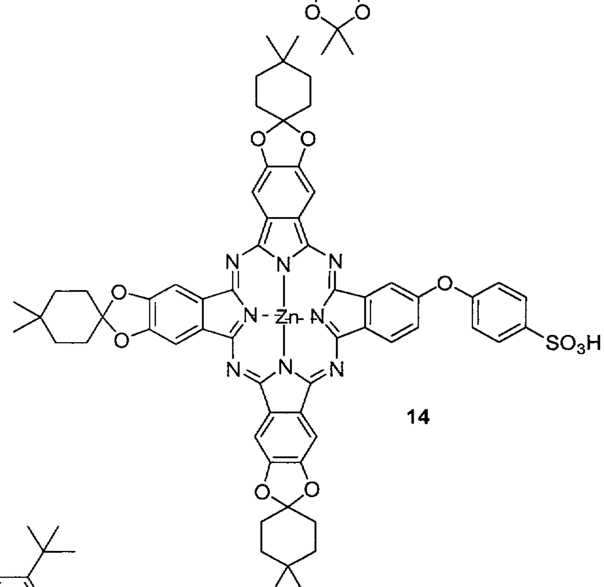


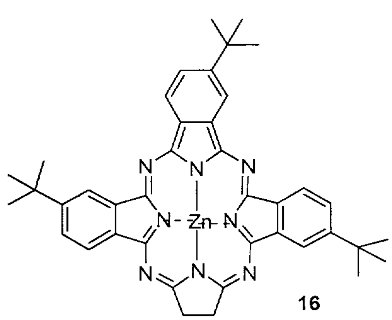
R = CO_2H **11**
R = SO_3H **13**



R = CO_2H **12**
R = SO_3H **15**



14



16

Synthesis of β-cyclodextrin dimers

Dimer **1** was synthesized as described previously (U.S. Serial No. 09/352,529, filed July 13, 1999, now allowed; Ruebner et al. 1999). Dimers **17** and **18** were prepared as illustrated in **Figure 2** using linker **19** attached to the primary and secondary faces of β-cyclodextrin respectively. To make **19**, disulfide **20** was reduced with sodium in ammonia, and then reacted with *cis*-1,2-dichloroethylene to afford diacid **19**. This was then coupled with 6-deoxy-6-amino-β-cyclodextrin, using dicyclohexylcarbodiimide (DCC) and hydroxybenzotriazole (HOBt), to afford **17**, and coupled with 3-deoxy-3-amino-β-cyclodextrin under the same conditions to afford **18**.

The 3-deoxy-3-amino-β-cyclodextrin was prepared as previously described (Yuan et al. 1998) by preparing the 3-naphthalenesulfonate of β-cyclodextrin, closing it to the 2,3-alloepoxide with base and opening this with sodium azide. Reduction with triphenylphosphine then afforded 3-deoxy-3-amino-β-cyclodextrin, which was coupled with **19** to afford **18**. The ^{1}H NMR spectrum of the azide showed that the attachment was on carbon 3 of the cyclodextrin, thus affording the product with overall retention of configuration. A small amount of the 2-azido compound was also formed, which was easily removed by chromatography.

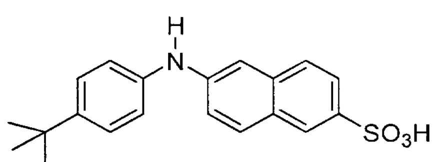
Finally, a β-cyclodextrin dimer with a shorter linker was synthesized, compound **21**, as shown in **Figure 3**. 2-Mercaptoethanol was coupled with *cis*-1,2-dichloroethylene, the hydroxyls then converted to bromides with triphenylphosphine dibromide, and the bromines replaced with potassium thioacetate. Then the acetate groups were removed with NaOMe, and the

dithiolate was directly coupled with 6-deoxy-6-iodo-β-cyclodextrin to afford **21.**

Binding studies

Estimates of likely binding pairs were obtained by MacroModel simulations of the dimers and the phthalocyanines. The distances between the carbons of the two β-cyclodextrins to which the linkers are attached are: **1**, 22 Å; **17**, 20 Å; **18**, 18 Å; **21**, 16 Å. For the phthalocyanines, distances were measured from the methylated carbons across the ring for **5**, 25 Å; **8**, 17 Å and **9**, 23 Å. For the adamantane derivative **10**, the distance across the entire system, including all the adamantane atoms, was 23 Å. The MacroModel simulations also suggested that the alkene in the linker would be positioned directly above the metal center when the phthalocyanine is bound into the dimer. This would be expected to facilitate desired site-specific oxidation.

Binding constants for a few of the possible pairs were determined by a fluorescence competition method previously used for cyclodextrin dimers, competing the substrate of interest with 2-(p-*tert*-butylanilino)naphthalene-6-sulfonic acid **22**, (termed BNS, Breslow et al. 1989).



22

BNS is fluorescent when bound into a hydrophobic cavity such as that of a cyclodextrin, but only

weakly fluorescent in water solution. Only the sulfonated phthalocyanines were soluble enough for this method.

The binding constant of BNS **22** to each dimer was determined as previously (Breslow et al. 1989, Ruebner et al. 1999) by titrating BNS into a dimer solution in a fluorescence cell, then exciting this solution at 330 nm and measuring the fluorescent emission at 438 nm. The binding constant of phthalocyanine to dimer was measured by titration of BNS into a 1:1 mixture of dimer and phthalocyanine. The double reciprocal plot of change in fluorescent intensity and BNS concentration gives straight lines (for an example of such a plot, see **Figure 4**). The straight lines found for each run show that the dimer and BNS were forming 1:1 complexes (Ruebner et al. 1996). The binding constant of BNS to each dimer is given by K = intercept/slope, and the binding constant of the phthalocyanine can be calculated as $K_I = (K/K' - 1)/[I]$, where K′ is the apparent binding constant of BNS to the dimer in presence of phthalocyanine, and [I] is the concentration of phthalocyanine (Ruebner et al. 1996).

The binding constants of BNS to the dimers used were as follows: **1**, 1.93×10^5 (reported 1.9×10^5, Ruebner et al. 1999); **17**, 1.07×10^6; **18**, 4.18×10^5; **21**, 7.01×10^5. For the dimers with the phthalocyanines: **1/2**, 2.00×10^6 (reported 2×10^6, Ruebner et al. 1999); **17/15**, 6.05×10^5; **18/14**, 1.94×10^6; **18/15**, 1.76×10^6; **21/13**, 1.30×10^6. All units in M^{-1} at ca. 25 °C.

As previously reported (Ruebner et al. 1999), there is some evidence that the linker chain in **1** is partly

tucked into one cyclodextrin cavity in water solution, so these binding constants are somewhat diminished as a result. In the ^{1}H NMR spectrum of **1**, the two vinyl protons are equivalent in DMSO solution, but non-equivalent in water. They become equivalent in water when hyodeoxycholic acid is added; this is known to bind strongly into β-cyclodextrin (Yang and Breslow 1997), and would thus be expected to displace the chain. Partial binding of the linker chain of **1** into one of the cyclodextrins in water leads to the non-equivalence, which apparently equilibrates slowly on the NMR time scale. This phenomenon was seen in the ^{1}H NMR spectra of all four of the cyclodextrin dimers described herein.

Photochemical cleavage

All of the photocleavage reactions were carried out using the same apparatus, as described previously (U.S. Serial No. 09/352,529, filed July 13, 1999, now allowed; Ruebner et al. 1999). A halogen lamp (50 W) was set up with a 540 nm cut-off filter and a focusing lens. The NMR tube in which the reactions were run was placed in the most strongly focused area, and oxygen was bubbled continuously through the solution while the photocleavage reactions were run. To monitor the reactions, ^{1}H NMR's were taken at regular intervals, whereby the disappearance of the alkene peaks and the appearance of the single formyl peak could be observed.

The dimers were at a concentration of 2.5 mM while the photosensitizers were at 0.14 mM in 5% DMSO-d_6 in D_2O. Typical ^{1}H NMR traces following the progress of a photocleavage reaction are shown in **Figure 5**. Here

dimer **21** was cleaved using phthalocyanine **11**. It can clearly be seen that the amount of alkene (doublet at ~6.2 ppm) decreases and the formyl peak (singlet at ~8.4 ppm) appears as the reaction progresses, and that no other peaks are seen in this area.

The plots of percent cleavage vs. time were all linear, indicating that the phthalocyanines remain active during the reactions and the light intensity is essentially constant. Furthermore, the NMR tubes were repeatedly removed from the apparatus, wrapped in aluminum foil, and taken to the NMR machine, then returned to the apparatus. The consistency of data indicates that the light flux was consistent throughout the runs. Also, repeats of the **21/13** experiment more than three weeks apart gave values of 5.3 and 5.4 min for 50% cleavage, showing that the photolysis apparatus is stable.

Comparison was made of the relative times needed for 50% cleavage of the dimers by various bound phthalocyanines under the conditions above, at ambient temperature (25 °C). The data are listed in Table 1.

Table 1. Times for 50% cleavage of the dimers (min)

	Phthalocyanine									
	2	**5**	**6**	**8**	**11**	**12**	**13**	**14**	**15**	**16**
Dimer **1**	7					60			6	
Dimer **17**		80	40							
Dimer **18**			55					6		
Dimer **21**			85	180	55		5.3; 5.4			22% in 180 min

The dimers were at a concentration of 2.5 mM while the photosensitizers were at 0.14 mM in 5% DMSO-d_6 in D_2O.

As the data indicate, there is considerable variation in the effectiveness of the photolytic cleavage process. Dimer **1** is rapidly cleaved by the previously made sulfonate sensitizer **2**, a mixture of eight isomers, and also by the well-defined adamantyl sulfonate sensitizer **15**, but not as rapidly by the corresponding carboxylate **12**. Dimer **17** was not rapidly cleaved by either **6**, the carboxylate version of **2**, or by **5**, the version of **2** with no solubilizing group.

The importance of a solubilizing group is clear. Sensitizers **5**, **8**, and **16** have no sulfonate or carboxylate group, and all their cleavage reactions are slow. Sensitizers **6**, **11**, and **12** have only carboxylate solubilizing groups, and are not as effective as the sulfonates in **2**, **13**, **14**, and **15**. As mentioned above, the high reproducibility of the data for the **21**/**13** cleavage reaction, data collected more

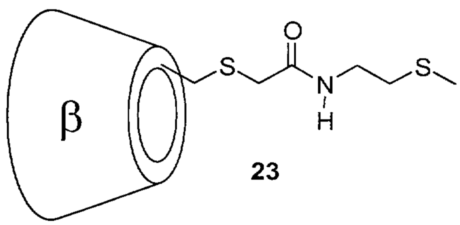
than three weeks apart, indicates that these data are reliable. Since the compounds were apparently in solution during these runs, it is likely that the better solubilizing groups produce a faster off rate once the linker is cleaved, permitting the turnovers that are involved in these processes with their excess of dimer over photosensitizer.

Good agreement of the experimental data with the MacroModel information can also be seen here. According to the MacroModel calculations, phthalocyanine **6** would be expected to bind most effectively with dimer **17** and least effectively with dimer **21**. This agrees very well with the experimental data, where the times for 50% cleavage of dimers **17**, **18** and **21** by phthalocyanine **6** are 40, 55 and 85 minutes respectively.

Control reactions were performed. When the oxygen was replaced by argon there was no cleavage, nor any in the absence of the sensitizer under the normal conditions. When the sensitizer was replaced by methylene blue, dimer **1** was cleaved to product **4** with its ^1H NMR peak at 8.4 ppm for the formyl group, but additional peaks were also seen at 10.2 and 7.8 ppm. Apparently singlet oxygen generated this way, rather than in complex **3**, is able to attack the cyclodextrin ring also. As pointed out previously (U.S. Serial No. 09/352,529, filed July 13, 1999, now allowed; Ruebner et al. 1999), this indicates that the singlet oxygen generated in the complex **3** is selectively taken up by the nearby olefin linkage of **1**. After dimer **21** had been completely cleaved by sensitizer **13**, in ca. 10 minutes, irradiation was continued for an additional 20 minutes but produced no further

change in the ^1H NMR of the solution. In accordance with this result, when the cyclodextrin dimer was replaced by β-cyclodextrin and the reaction was run under normal conditions, no oxidation products were observed.

The cleavage of dimers diminishes their affinity for the sensitizers, and not just because the chelate effect is gone. Examination was made of the binding of compound **23**, which mimics the cleavage product **4**, except with a methyl group replacing the somewhat hydrolytically labile formyl group.



23

With 4-*tert*-butylbenzoic acid, β-cyclodextrin has a binding constant of 1.7×10^4 M^{-1}, while **23** had a binding constant of only 1.8×10^3 M^{-1}, an order of magnitude less. This difference can be ascribed to the competitive binding of the chain in **23** into the cyclodextrin cavity. It is proposed that the same interaction occurs with cleavage product **4** and related cleavage products from the other dimers.

Concentration of the sensitizer complex into a light beam was achieved using an experiment described previously (Ruenber et al. 1999) in which a tube was shielded with aluminum foil so that only a small section could be irradiated through a window in the foil. Phthalocyanine **13** and dimer **21** were made up in a 1:1 solution in D_2O. A small amount of **13** was insoluble, and removed. The solution was then

irradiated through the window, and after 40 hours sensitizer **13** had precipitated solely in the window, and the ^{1}H NMR of the solution indicated that all the dimer **21** had been cleaved; all the vinyl protons were gone, replaced by formyl protons of the monomer. Thus, as expected, the dissolved components do diffuse into the light beam, where they undergo the cleavage reaction.

<u>Characterization of the dimers and the phthalocyanines</u>.

Dimer **1:** ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.09 (t, 2H), 6.22 (s, 2H), 5.90-5.60 (m, 28H), 4.95-4.75 (m, 14H), 4.55-4.40 (m, 12H), 3.90-3.45 (m, 56H), 2.77 (t, 4H); MS (FAB): 2560 ($M+2^{+}$, 10).

Dimer **17:** ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.68 (bs, 2H), 6.16 (s, 2H), 5.80-5.67 (m, 28H), 4.81 (m, 14H), 4.47 (m, 14H), 3.62-3.32 (m, 84H), 2.72-2.66 (m, 4H), 2.21-2.10 (m, 4H), 1.84-1.66 (m, 4H); MS (MALDI) *m/z* (%) 2517 ($M+1+Na^{+}$, 5).

Dimer **18:** ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.90 (bs, 2H), 6.20 (s, 2H), 5.99 (d, *J* = 6.1, 2H), 5.79-5.61 (m, 26H), 4.82-4.75 (m, 14H), 4.50-4.45 (m, 14H), 3.62-3.32 (m, 84H), 2.73 (m, 4H), 2.26-2.23 (m, 4H), 1.82-1.79 (m, 4H); MS (MALDI) *m/z* (%) 2516 ($M+Na^{+}$, 15).

Dimer **21:** TLC R_f 0.13 7:7:5 i-PrOH:Ethyl Acetate:Water; ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.24 (s, 2H), 5.78-5.67 (m, 28H), 4.84 (s, 14H), 4.49-4.43 (m, 12H), 3.87-3.30 (m, 80H), 3.05-2.64 (m, 12H).

Zinc phthalocyanine **2:** TLC R_f 0.24 20% MeOH (10% NH_3):$CHCl_3$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.45-9.30 (m,

4H), 8.92-8.73 (m, 4H), 7.89-7.81 (m, 3H), 7.70-7.61 (m, 8H), 7.45-7.35 (m, 8H), 1.47-1.41 (m, 27H)); MS (FAB) *m/z* (%) 1193 ($M+H^+$, 5); λ_{max} (nm) 678.

Zinc phthalocyanine **5**: TLC R_f 0.67 10% MeOH (10% NH_3):$CHCl_3$; ^1H NMR (300 MHz, $CDCl_3$) δ 8.72-7.78 (m, 8H), 7.60-7.51 (m, 4H), 7.38-7.07 (m, 16H), 1.55-1.42 (m, 36H); MS (FAB) *m/z* (%) 1170 ($M+H^+$, 2); λ_{max} (nm) 680.

Zinc phthalocyanine **6**: ^1H NMR (300 MHz, $CDCl_3$) δ 9.22-8.60 (m, 8H), 8.25-7.35 (m, 20H), 1.46-1.35 (m, 27H); MS (FAB) *m/z* (%) 1157 ($M+H^+$, 5).

Zinc phthalocyanine **8**: ^1H NMR (300 MHz, $CDCl_3$) δ 7.15-7.05 (m, 8H), 1.81-1.73 (m, 24H); MS (APCI) *m/z* (%) 864 (M^+, 4); λ_{max} (nm) 667.

Zinc phthalocyanine **9**: ^1H NMR (300 MHz, $CDCl_3$) δ 8.15 (bs, 8H), 2.35 (bs, 16H), 1.88 (bs, 16H), 1.24 (s, 26H); MS (FAB) *m/z* (%) 1137 ($M+H^+$, 6), 1136 (M^+, 5); λ_{max} (nm) 669.

Zinc phthalocyanine **10**: TLC R_f 0.80 10% MeOH (10% NH_3):$CHCl_3$; 1H NMR (300 MHz, $CDCl_3$) δ 8.60 (bs, 8H), 1.61-2.67 (m, 56H); MS (FAB) m/z (%) 1235 (M+H+, 5); λ_{max} (nm) 668.

Zinc phthalocyanine **11**: TLC R_f 0.24 20% MeOH (10% NH_3):$CHCl_3$; 1H NMR (300 MHz, DMSO-d_6) δ 8.71 (m, 4H), 8.13-8.01 (m, 5H), 7.71-7.23 (m, 4H), 5.76 (s, 1H), 1.96 (s, 18H); MS (FAB) m/z (%) 930 ($M+H^+$, 2) 929 (M^+, 2); λ_{max} (nm) 666.

Zinc phthalocyanine **12**: TLC R_f 0.25 20% MeOH (10% NH_3):$CHCl_3$; ^1H NMR (300 MHz, DMSO-d_6) δ 9.38-9.33 (m, 1H), 8.94-8.90 (m, 1H), 8.58-8.42 (m, 4H), 8.16 (d, *J* = 8.4, 2H), 8.04-7.92 (m, 1H), 7.58 (s, 1H), 7.49 (d, *J* = 8.7, 2H), 7.27 (s, 1H), 2.33-1.73 (m, 42H); MS (FAB) *m/z* (%) 1205 (M^+, 0.5); λ_{max} (nm) 667.

Zinc phthalocyanine **13**: TLC R_f 0.20 20% MeOH (10% NH_3):$CHCl_3$; ^1H NMR (300 MHz, DMSO- d_6) δ 9.42-9.36 (m, 1H), 8.72-8.50 (m, 4H), 8.41-8.21 (m, 1H), 7.78 (d, *J* = 8.7, 2H), 7.64-7.53 (m, 1H), 7.32 (d, *J* = 9.0, 2H), 7.25 (s, 1H), 7.11 (s, 1H), 1.98-1.95 (m, 6H), 1.71-1.65 (m, 12H); MS (FAB) *m/z* (%) 966 ($M+H^+$, 1.5), 965 (M^+, 1.5); λ_{max} (nm) 665.

Zinc phthalocyanine **14**: TLC R_f 0.21 20% MeOH (10% NH_3):$CHCl_3$; ^1H NMR (300 MHz, DMSO-d_6) δ 9.38-9.28 (m, 1H), 8.89-8.82 (m, 1H), 8.67-8.49 (m, 4H), 7.82 (d, *J* = 7.5, 2H), 7.37 (d, *J* = 8.1, 2H), 7.25 (d, *J* = 7.2, 1H), 7.03 (s, 1H), 6.87 (d, *J* = 7.5, 1H), 2.27 (bs, 4H), 1.96 (bs, 4H), 1.74 (bs, 4H), 1.49 (bs, 4H), 1.22-0.74 (m, 26H); MS (FAB) *m/z* (%) 1171 (M^+, 30); λ_{max} (nm) 666.

Zinc phthalocyanine **15**: TLC R_f 0.25 20% MeOH (10% NH_3):$CHCl_3$; ^1H NMR (300 MHz, DMSO-d_6) δ 9.378 (bs, 2H), 8.93-8.85 (m, 1H), 8.70-8.52 (m, 3H), 7.80 (d, *J* = 7.5, 2H), 7.54 (s, 1H), 7.50 (d, *J* = 9.9, 1H), 7.37 (d, *J* = 7.5, 2H), 7.05 (s, 1H), 2.26-1.72 (m, 42H); MS (FAB) *m/z* (%) 1243 (M^+,10); λ_{max} (nm) 667.

Details of Binding studies

Monomer **23** with p-*tert*-butylbenzoic acid

β-cyclodextrin (CD) monomer **23** (6.4 mg, 0.005 mmol) was dissolved in 0.20 M pH 9.0 Na_2CO_3/$NaHCO_3$ buffer (5.00 mL) to make a 1.0 mM solution. p-*tert*-Butylbenzoic acid (11.0 mg, 0.062 mmol) was dissolved in 6.16 mL of the same buffer to make a 10.0 mM solution. Both solutions were degassed under reduced pressure with a sonicator for 5 minutes immediately prior to the binding study. The β-CD monomer **23** solution (2.50 mL) was put into the sample cell compartment of an Omega microcalorimeter, whereas the p-*tert*-butyl-benzoic acid was loaded in a 250 μL syringe and then assembled onto the calorimeter. The system was equilibrated until RMS error was less than 5×10^{-3} with the syringe spinning at 400 rpm. The p-*tert*-butylbenzoic acid solution was then injected into the cell in 25 injections (10 μL, 7 seconds per injection). The time interval between injections was set to be 4 minutes. Injection data were automatically collected by the computer, and the data was analyzed by ORIGIN software with the single-binding-site model. Two trials were performed.

	Trial 1	Trial 2
Binding constant:	$1.8 \pm 0.2 \times 10^3$ M^{-1}	$1.8 \pm 0.2 \times 10^3$ M^{-1}
Binding ratio:	0.91 ± 0.07	0.99 ± 0.07

Binding of β-cyclodextrin to p-*tert*-butylbenzoic acid

β-Cyclodextrin (4.0 mg, 0.0035 mmol) was dissolved in 0.20 M pH 9.0 Na_2CO_3/ $NaHCO_3$ buffer (3.53 mL) to make a 1.0 mM solution. p-*tert*-butyl-benzoic acid (10.0 mg, 0.056 mmol) was dissolved in 5.60 mL of the same buffer to make a 10.0 mM solution. Both solutions

were degassed under reduced pressure with a sonicator for 5 minutes immediately prior to the binding study. The β-Cyclodextrin solution (2.50 mL) was put into the sample cell compartment of the microcalorimeter, whereas the p-*tert*-butyl-benzoic acid was loaded in a 250 μL syringe and then assembled onto the calorimeter. The whole setup was equilibrated until RMS error was less than $5x10^{-3}$ with the syringe spinnning at 400 rpm. The p-*tert*-butyl-benzoic acid solution was then injected into the cell in 25 injections (10 μL, 7 seconds per injection). The time interval between injections was set to be 4 minutes. Injection data were automatically collected by the computer, and the data was analyzed by ORIGIN software with the single-binding-site model. Binding constant: $1.7 \pm 0.2 \times 10^4\ M^{-1}$.

<u>Representative binding study of a phthalocyanine to a cyclodextrin dimer</u>.

Three solutions were made:

i) β-cyclodextrin dimer **21** (0.20 mg, 8.18×10^{-8} mol) was dissolved in degassed water (250 mL).

ii) BNS **22** (0.14 mg, 3.94×10^{-7} mol) was dissolved in degassed water (4.00 mL).

iii) β-cyclodextrin dimer **21** (0.40 mg, 1.64×10^{-7} mol) and phthalocyanine **13** (1.64×10^{-7} mol) were dissolved in a mixture of methanol (1 mL) and water (0.1 mL). This mixture was stirred in the dark for 1 h. The solution was then concentrated under vacuum and then placed under high-vacuum for 18 h. The resulting material was dissolved in degassed water (500 mL).

The binding constant for BNS to cyclodextrin dimer **21** was determined by the fluorescence emission method. Cyclodextrin dimer **21** solution (3.00 mL) was added to a fluorescence cell and was excited at 330 nm and the emission at 418 nm was measured. Five additions of BNS solution (10 μL) were made, with a measurement being taken after each one. The area under the peak between 400 and 500 nm was measured for each addition. The experiment was run in duplicate.

The binding constant for phthalocyanine **13** to dimer **21** was determined by the fluorescence emission method. 1:1 Dimer: phthalocyanine solution (3.00 mL) was added to a fluorescence cell. This solution was excited at 330 nm and the emission at 418 nm was measured. Five additions of BNS solution (10 μL) were made, with a measurement being taken after each one. The experiment was run in duplicate. The data are plotted in **Figure 4**.

Representative photocleavage procedure

To a solution of β-cyclodextrin dimer **17** (5.9 mg, 2.35×10^{-6} mol) and potassium carbonate (2 mg) in D_2O (1.00 mL) was added a 3 mM solution of phthalocyanine **6** (0.17 mg, 1.50×10^{-7} mol) in DMSO-d_6 (50 μL). This solution was transferred to an NMR tube and was irradiated with a halogen lamp (50 W) with a cut-off filter to exclude wavelengths below 540 nm. During irradiation, oxygen was bubbled through the solution. The reaction was monitored by ^{1}H NMR, by observance of the disappearance of the alkene peaks and the appearance of the formyl peak. Whenever the ^{1}H NMR was taken, the NMR tube was completely shielded from light using an aluminum foil cover during

transportation to the NMR room. The results of a typical run are shown in **Figure 5**.

Representative precipitation experiment procedure

β-Cyclodextrin dimer **21** (2.4 mg, 1.00 x 10^{-6} mol), potassium carbonate (0.5 mg) and phthalocyanine **13** (0.97 mg, 1.00 x 10^{-6} mol) were dissolved in a mixture of methanol (1 mL) and D_2O (0.1 mL). The mixture was stirred in the dark for 1 h. The solvents were removed under vacuum and the resulting solid was placed under high vacuum for 18 h. The residue was treated with D_2O (1.00 mL) and stirred in the dark for 2 h, during which time everything appeared to have dissolved. The solution was then filtered through a cotton wool plug and transferred to an NMR tube. The tube was covered in aluminum foil except for an area approximately 0.5 cm wide, which was left uncovered, through which the solution could be seen. Oxygen was bubbled through the solution for 5 min while the solution was kept in the dark. The solution was then placed on its side and was irradiated with a halogen lamp (50 W) with a cut-off filter to exclude wavelengths below 540 nm. The solution was re-saturated with oxygen, in the same manner as above, after 18 h.

Detailed Synthesis

In the Detailed Synthesis section only, the numbering of structures differs from that in the remainder of the application since many more numbered structures are included here. The corresponding numbers are as follows: remainder of application:(Detailed Synthesis) - 1:(2), 2:(1), 5:(14), 6:(17), 7:(22),

8:(24), 9:(29), 10:(34), 11:(35), 12:(36), 13:(39), 14:(40), 15:(41), 16:(42), 17:(43), 18:(44), 19:(45), 20:(46), 21:(67), 22:(74), 23:(71).

Solvents, drying reagents and inorganic salts were obtained from Aldrich Chemical Company or Fisher Scientific Company and used without further purification unless otherwise specified. β-cyclodextrin was obtained from American Maize Company. THF and CH_2Cl_2 were dried by distillation under argon from Na.Benzophenone and calcium hydride respectively. Anhydrous DMF was obtained from Aldrich in SureSeal™ bottles. Argon was obtained from Matheson.

^{1}H NMR spectra were recorded on Bruker DMX 300, 400 or 500 MHz or Varian VXR 400 MHz instruments with the residue solvent peaks as the reference signal. TMS (tetramethyl silane) was used as internal reference for the measurements in $CDCl_3$. ^{13}C spectra were recorded on Varian VXR 300 MHz or Bruker DMX 300 MHz instruments. All chemical shifts were reported in parts per million (ppm) downfield of zero on the delta (δ) scale.

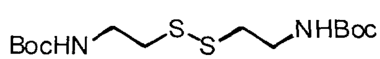
Mass spectra were recorded on a Nermag R-10-10 spectrometer for CI and EI spectra, or a Jeol JMS-DX-303 HF instrument for FAB spectra. Infrared spectra were recorded on a Perkin-Elmer 1600 Fourier Transform spectrometer. Ultraviolet/Visible (UV-vis) spectra were recorded on a Varian CARY 1E spectrometer. Microcalorimetic titrations were performed on an OMEGA calorimeter. Melting points were determined using MelTemp capillary melting

point apparatus and were uncorrected. Cyclodextrin products were dried by a VirTis Sentry Lyophiliser.

Analytical thin layer chromatography (TLC) was performed on 0.25 mm precoated silica gel plates with 254 nm fluorescence indicator from EM science. Compounds were visualised under UV light or by TLC staining solutions. All cyclodextrin compounds were stained with anisaldehyde stain (a solution of p-anisaldehyde (9.2 mL), glacial acetic acid (3.7 mL) and concentrated H_2SO_4 (12.5 mL) in 190 proof ethanol (340 mL), and then heated until the blue-gray spots appear.

Silica gel column chromatography was performed with 230-400 mesh silica from EM science. All reverse phase column chromatography was performed using homemade C-18 reverse phase silica gel. The compounds containing β-cyclodextrins were dissolved in water and loaded onto the column, then eluted with the linear gradient of solvent systems described.

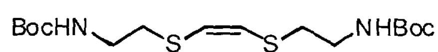
All reactions were performed under an atmosphere of argon unless otherwise specified.



7

Cystamine (4.00 g, 17.7 mmol) was dissolved in a mixed solvent system of dioxane/water (1:1) (30 mL), followed by the addition of sodium hydroxide (1.40 g, 35.4 mmol). Boc_2O (8.20 g, 38.8 mmol) was added after the solution was cooled to 0 °C. The solution was warmed to 25 °C and stirred for 30 min. After a few minutes a precipitate appeared. The solvent was

removed under reduced pressure and the residue was dissolved in ethyl acetate. The insoluble parts (salts) were removed by filtration, and the filtrate was washed with 1 M HCl (10 mL), water (10 mL), and 1 M NaOH (10 mL), then dried ($MgSO_4$). Concentration of the solution gave [2-(2-*tert*-butyoxycarbonylamino-ethyldisulfanyl)-ethyl]carbamic acid *tert*-butyl ester **7** (6.40 g, 86%) as a white solid. Mp 105 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ6.95 (t, J = 5.4, 2H), 3.16-3.23 (m, 4H), 2.51 (t, J = 7.0, 4H), 1.37 (s, 18H); ^{13}C NMR δ 155.5, 77.7, 37.6, 28.2; MS (APCI) m/z (%) 353 (M+H$^+$, 20).



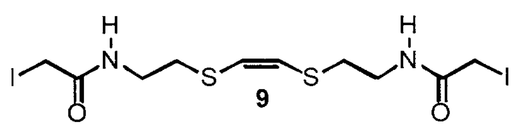
8

Diamide **7** (2.00 g, 5.60 mmol) was placed in a small 3-necked-flask which was evacuated and backfilled with argon 3 times. Ammonia (30 mL) was condensed into the flask using a cold trap. Pieces of sodium metal were added to the flask until the blue colour remained. The solution was stirred for 30 minutes, and more sodium was added if the blue colour disappeared. A minimum amount of solid ammonium chloride was added to quench the reaction until the solution became colourless. *cis*-1,2-Dichloroethylene (0.41 mL, 5.50 mmol) was added with a syringe. The reaction was stirred for 4 hours and then the ammonia was evaporated at 25 °C. The remaining solid was dissolved in a mixture of water (10 mL) and ethyl acetate (10 mL). The ethyl acetate phase was separated, washed with water (10 mL), dried ($MgSO_4$) and concentrated to afford {2-[2-(2-*tert*-butyoxycarbonylamino-ethylsulfanyl)-vinylsulfanyl]-ethyl}carbamic acid *tert*-butyl ester **8** (2.2g, yield=

96%). The product was pure enough for further reactions. Mp 124 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.95 (t, 2H), 6.20 (s, 2H), 3.06-3.12 (m, 4H), 2.74 (t, J = 7.5, 4H), 1.36 (s, 9H); ^{13}C NMR δ 155.4, 123.0, 77.8, 32.6, 28.2; MS (APCI) m/z (%) 379 ($M+H^+$, 50).

5

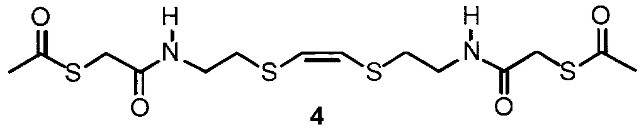
To a solution of the Boc protected linker **8** (0.50 g, 1.30 mmol) in dioxane (8 mL) was added a solution of HCl in dioxane (10 mL) and the solution was stirred at 25 °C for 1 h. After filtration, the crude product was dissolved in methanol, and precipitated with CH_2Cl_2. The product was filtered and dried to give 2-[2-(2-amino-ethylsulfanyl)-vinylsulfanyl]-ethylamine **5** as the di-hydrochloric acid salt (0.28 g, 86%) as a white solid. Mp 155 °C; ^{1}H NMR (300 MHz, MeOH-d_4) δ 6.31 (s, 2H), 3.15 (t, J = 7.0, 4H), 3.01 (t, J = 7.5, 4H); ^{13}C NMR δ 124.9, 40.6, 31.7; MS (APCI) m/z (%) 179 ($M+H^+$, 65).



9

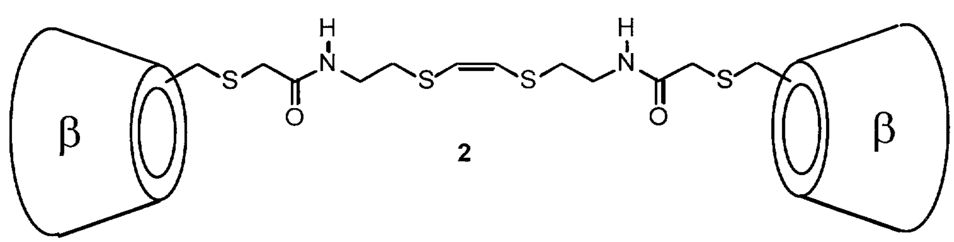
To a solution of diamine **5** (0.20 g, 0.80 mmol) in 0.1 M sodium hydroxide aqueous solution (40 mL) was added a solution of iodoacetic anhydride (0.78 g, 2.20 mmol in 1,2-dichloroethane (10 mL). The mixture was vortexed for 2 minutes. The product was formed as a white precipitate and was filtered out by a frit funnel. The solid was washed with water (10 mL) and dried to produce 2-iodo-*N*-(2-{2-[2-(2-iodo-acetylamino)-ethylsulfanyl]vinylsulfanyl}-ethyl)-acetamide **9** (0.34 g, 85%) as a white solid. Mp 129 °C

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.45 (bs, 2H), 6.25 (s, 2H), 3.64 (s, 4H), 3.25 (m, 4H), 2.88 (t, J = 6.3, 4H).



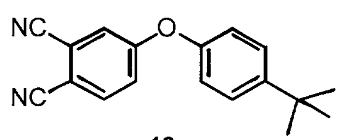
4

A solution of diamide **9** (0.22 g, 0.43 mmol) and potassium thioacetate (0.11 g, 0.95 mmol) in methanol (80 mL) was evacuated and purged with argon for three times, and then stirred at 50 °C for 4 hours. The methanol was removed under vacuum and the residue was extracted with ethyl acetate (20 mL) and water (20 mL). The organic phase was washed with water (10 mL), dried ($MgSO_4$), and concentrated to give thioacetic acid *S*-[(2-{2-[2-(2-acetylsulfanyl-acetylamino)-ethylsulfanyl]-vinylsulfanyl}-ethylcarbamoyl)-methyl] ester **4** (0.15 gm, 84%) as a white solid. Mp 129 °C; ^{1}H NMR (300 MHz, MeOH-d_4) δ 6.18 (s, 2H), 3.61 (s, 4H), 3.37 (t, J = 6.8, 4H), 2.80 (t, J = 7.0, 4H), 2.46 (s, 6H); ^{13}C NMR δ 195.5, 168.3, 124.7, 39.6, 33.6, 33.0, 30.3; MS (APCI) m/z (%) 411 (M+H$^+$, 100).



2

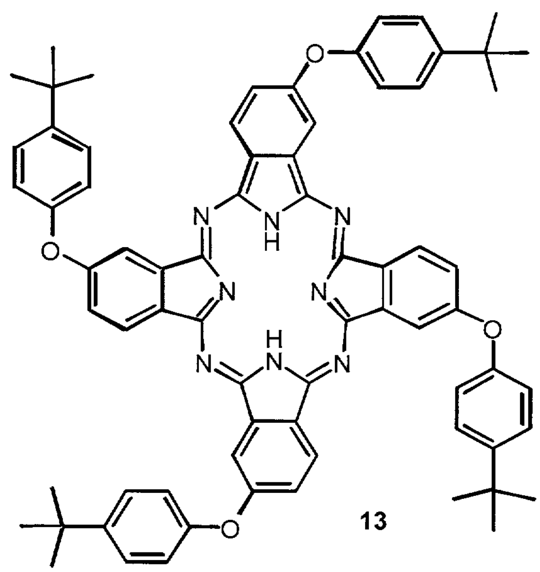
Dithioacetate **4** (0.20 g, 0.58 mmol) and potassium hydroxide (0.10 g, 1.79 mmol) were dissolved in methanol (100 mL). The solution was evacuated and purged with argon three times. The solution was stirred at 50 °C for 10 minutes and then the methanol was removed under vacuum. The residue was dissolved in DMF (40 mL) and 6-monoiodo-β-cyclodextrin (1.00 g, 8.04 mmol) was added. The reaction was stirred at 50 °C for 18 h, then poured into acetone (1 L). The resultant precipitate was filtered and purified by reverse-phase column using a H_2O/MeOH solvent gradient (MeOH 20-80%). This gave pure dimer **2** (0.23 g, 22%) as a white solid. ^1H NMR (300 MHz, DMSO-d_6) δ 8.09 (t, 2H), 6.22 (s, 2H), 5.90-5.60 (m, 28H), 4.95-4.75 (m, 14H), 4.55-4.40 (m, 12H), 3.90-3.45 (m, 56H), 2.77 (t, 4H); MS (FAB): 2560 ($M+2^+$, 10).



12

To a solution of 4-*tert*-butylphenol **10** (2.26 g, 15.0 mmol) in dry DMSO (15 mL) was added 4-nitrophthalonitrile **11** (1.30 g, 7.50 mmol) and potassium carbonate (2.07 g, 15.0 mmol). The reaction was stirred for 20 h at 25 °C. The crude product was precipitated by pouring the solution into 150 mL of cold dilute HCl (5 mL of 37% HCl in 150 mL of water), filtered and washed with water until the washings were neutral pH. The crude product was

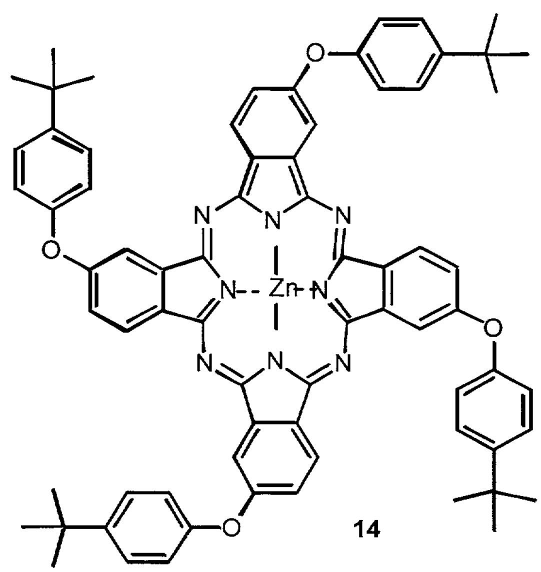
dissolved in CH_2Cl_2 (100 mL) and washed with 5% NaOH solution (5 x 75 mL) and water (75 mL). The resulting solution was dried ($MgSO_4$), filtered and concentrated to give 4-(4-*teret*-butyl-phenoxy)-phthalonitrile **12** (1.52 g, 73%) as a white solid. TLC R_f 0.22 1:4 Ethyl acetate:hexanes; Mp 122°C (lit.[x] 120°C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72 (dd, *J* = 7.3, 1.9, 2H), 7.47 (dd, *J* = 6.7, 2.2, 2H), 7.27 (s, 1H), 7.24 (d, *J* = 2.6, 1H), 7.00 (dd, *J* = 6.7, 1.1, 2H), 1.36 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.1, 151.1, 149.4, 135.4, 127.5, 121.4, 121.3, 120.1, 117.5, 115.5, 115.1, 108.5, 34.6, 31.4; MS (APCI) *m/z* (%) 277 ($M+H^+$, 5).



13

To a solution of lithium pentyloxide (prepared from reaction of lithium (25 mg) with pentanol (4.0 mL), at 140 °C was added dinitrile **12** (0.25 g, 0.91 mmol). The reaction mixture was heated to 140 °C for 3 h.

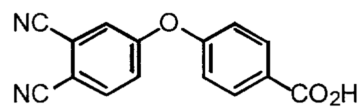
The mixture was allowed to cool and the solution was concentrated The resulting dark mass was dissolved in DMF containing a small amount of methanolic KOH (10 mL) and poured into acetone (10 mL). The precipitate was filtered, treated with concentrated HCl (10 mL), dissolved in acetone (75 mL) and treated with dilute ammonia (75 mL). The resulting precipitate was filtered and dried to afford pure phthalocyanine **13** (57 mg, 23%) as a blue/green solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.81-8.67 (m, 4H), 8.63-8.39 (m, 4H), 7.63-7.54 (m, 10H), 7.45-7.33 (m, 7H), 7.29-7.24 (m, 3H), 1.48-1.43 (m, 36H), 3.00 (bs, 2H); MS (FAB) *m/z* (%) 1107 ($M+H^+$,.0.5)



14

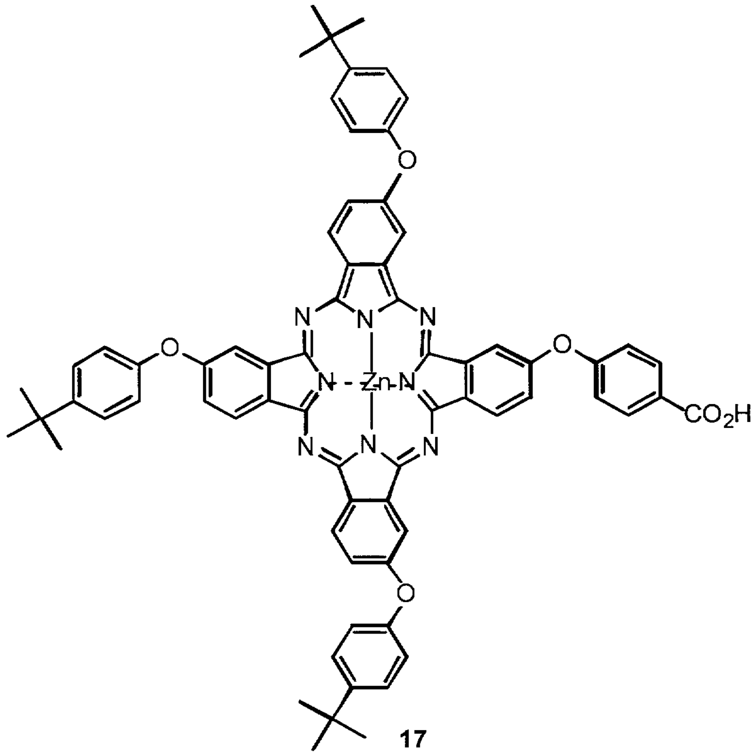
A mixture of phthalocyanine **13** (57 mg, 5.15×10^{-5}mol) and zinc acetate dihydrate (25 mg, 1.14×10^{-4}mol) in DMF (7 mL) was heated at 70 °C for 20 h. The DMF was removed under vacuum and the resulting solid washed with water (10 mL). TLC analysis (0.2% MeOH (10% NH_3):$CHCl_3$) showed there to be no other compounds in

the resulting solid. The product **14** was isolated as a blue/green solid (60 mg, 100%). TLC R_f 0.67 10% MeOH (10% NH_3):$CHCl_3$; ^1H NMR (300 MHz, $CDCl_3$) δ 8.72-7.78 (m, 8H), 7.60-7.51 (m, 4H), 7.38-7.07 (m, 16H), 1.55-1.42 (m, 36H; MS (FAB) *m/z* (%) 1170 ($M+H^+$, 2)); λ_{max} (nm) 680.



16

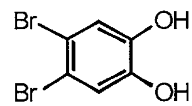
To a suspension of K_2CO_3 (3.50 g, 25 mmol) in dry DMSO (30 mL) was added 4-nitrophthalonitrile **11** (2.00 g, 11.5 mmol) and p-hydroxybenzoic acid (2.36 g, 17.1 mmol). Further K_2CO_3 (3.50 g, 25 mmol) were added after 3 h and after 24 h. The suspension was stirred at 25 °C for 5 days. The suspension was added to water (600 mL) and the pH was adjusted to 1 using concentrated HCl. The resulting precipitate was recrystallised from methanol (50 mL) to give pure 4-(3,4-dicyano-phenoxy)-benzoic acid **16** (2.65 g, 87%) as a white solid. ^1H NMR (300 MHz, $CDCl_3$) δ 8.16 (d, *J* = 8.7, 1H), 8.03 (d, *J* = 8.8, 2H), 7.94 (d, *J* = 2.5, 1H), 7.54 (dd, *J* = 8.7, 2.5, 1H), 7.27 (d, *J* = 8.8, 2H); MS (APCI) *m/z* (%) 297 ($M+MeOH^+$, 60).



17

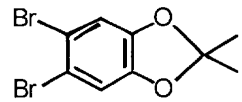
To a suspension of benzoic acid dinitrile **16** (0.19 g, 0.73 mmol) and *tert*-butyl dinitrile (0.30 g, 1.08 mmol) in pentanol (10 mL) at 140 ^{0}C was added lithium (0.10 g, 14.5 mmol) and the mixture was stirred at 140°C for 15 min. Once cooled to 25 ^{0}C, glacial acetic acid (30 mL) was added, the resultant precipitate was centrifuged and washed with water (10 mL). The products were dissolved in DMF (30 mL), zinc acetate dihydrate (0.12 g, 0.55 mmol) was added and the reaction was heated at 80 $^{\circ}$C for 15 h. The solution was cooled to 25 $^{\circ}$C, the DMF was removed under vacuum and the resulting solid was washed with water (20 mL). This solid was dissolved in the minimum volume of DMF and was purified by column chromatography on silica, starting with an eluent of

diethyl ether:DMF 98:2. Once the first fraction, the tetra-*tert*-butyl phthalocyanine **14**, had come off, the eluent was very slowly changed to pure DMF. The next fraction to come off was the desired complex **17**, which was isolated as a blue/green solid (88 mg, 21%). ^{1}H NMR (300 MHz, $CDCl_3$) 9.22-8.60 (m, 8H), 8.25-7.35 (m, 20H), 1.46-1.35 (m, 27H); MS (FAB) *m/z* (%) 1157 ($M+H^+$, 5).



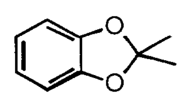
19

To a solution of catechol **18** (11.00 g, 0.10 mol) in glacial acetic acid (50 mL) at 0 °C was very slowly added a solution of bromine (11 mL, 34 g, 0.21 mol) in glacial acetic acid (50 mL). This mixture was stirred at 25 °C for 18 h and after this TLC (60:40 Hexane:Ethyl Acetate) suggested that there was little starting catechol **18** left. The HBr and glacial acetic acid were removed by distillation under water-pump vacuum. The dark residue was quenched with ice water (350 mL). The resulting precipitate was filtered, dried in vacuum and recrystallised from benzene to give three crops of crystals, giving 4,5-dibromocatechol **19** (15.37 g, 57%) as a white solid. TLC R_f 0.34 2:3 Ethyl acetate:hexanes; Mp 110 - 111°C (lit.[x] 119-121°C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.14 (s, 2H), 5.30 (s, 2H) ^{13}C NMR (75 MHz, $CDCl_3$) δ ; MS (APCI) *m/z* (%) 265 ($M-H^+$, 25).



20

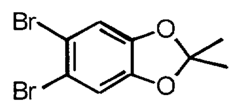
To a mixture of 4,5-dibromocatechol **19** (10.72 g, 0.04 mol) and P_2O_5 (1.14 g, 8.00 mmol) in toluene (20 mL) heated at 75 °C was added dropwise acetone (5.90 mL, 0.08 mol). During the addition, every 30 min P_2O_5 (1.14 g, 8.00 mmol) was added, for 2 h (4 portions). The mixture was then stirred for 1 h. Once cooled to 25 °C, the viscous oil was poured into 25% NaOH in water (25 mL), after which a white precipitate was seen to form. This precipitate was dissolved by addition of water (50 mL) to the solution. The aqueous layer was washed with water (3 x 50 mL), dried ($MgSO_4$) and concentrated to yield pure 5,6-dibromo-2,2-dimethyl-benzo[1,3]dioxole **20** (1.13 g, 9%) as a white solid. TLC R_f 0.63 1:4 Ethyl acetate:hexanes; Mp 93 - 94 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 6.97 (s, 2H), 1.66 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 147.9, 120.2, 114.7, 113.1, 25.9.



21

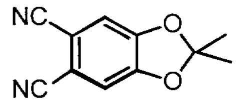
To a mixture of catechol **18** (11.00 g, 0.10 mol) and P_2O_5 (2.84 g, 20 mmol) in toluene (50 mL) at 75 °C was added dropwise acetone (14.7 mL, 0.2 mol) over 2 h. After the addition had been started, P_2O_5 (2.84 g, 20 mmol) was added to the reaction mixture every 30 min, total 3 portions. After 2 h, further acetone (7.3 mL, 0.1 mol) was added dropwise. The reaction was then stirred for a further 1 h and was then cooled to

25 °C. The organic solution was carefully decanted and to this was added 25% NaOH in water (15 mL). The organic layer was separated, washed with water (2 x 20 mL) and concentrated. The resulting oil was distilled under high vacuum to give 1,2-isopropylidenedioxybenzene (2,2-dimethyl-benzo[1,3]dioxole) **21** (5.00 g, 33%) as a clear liquid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.80-6.72 (m, 4H), 1.67 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 147.4, 121.1, 117.4, 108.5, 25.9; MS (APCI) *m/z* (%) 265 ($M-H^+$, 25).



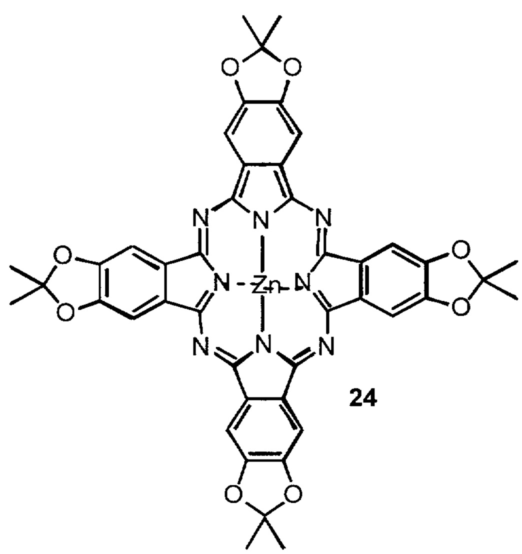
20

To a solution of 1,2-isopropylidenedioxybenzene **21** (4.80 g, 31.9 mmol) in DMF (60 mL) was added portionwise N-bromosuccinimide (11.94 g, 67.1 mmol) at 25 °C. The light yellow solution was stirred at 25 °C for 24 h. The solution was then poured into water (600 mL) and extracted with CH_2Cl_2 (7 x 150 mL). The combined organic extracts were washed with water (4 x 300 mL), dried ($MgSO_4$), filtered and concentrated. The resulting solid was recrystallised from warm benzene to give pure 5,6-dibromo-2,2-dimethyl-benzo[1,3]dioxole **20** (4.94 g, 50%) as a white solid. TLC R_f 0.63 1:4 Ethyl acetate:hexanes; Mp 93 - 94 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.97 (s, 2H), 1.66 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 147.9, 120.2, 114.7, 113.1, 25.9.



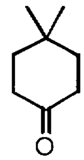
22

A mixture of **20** (4.94 g, 16.0 mmol), CuCN (5.77 g, 654.2 mmol) in dry DMF (65 mL) was heated to 155 °C for 5h. The dark mixture was treated with ammonia water (250 mL) and stirred for 30 min. The mixture was then filtered, washed with water (100 mL) and dried in air for 18 h. The resulting solid was extracted with diethyl ether using a Soxlet extractor for 3 days. The solvent was then removed and the residue twice recrystallised from benzene to give 2,2-dimethyl-benzo[1,3]dioxole-5,6-dicarbonitrile **22** (1.58 g, 49%) as a white solid. Mp 202 - 203 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.06 (s, 2H), 1.77 (s, 6H; MS (APCI) *m/z* (%) 265 ($M+MeOH^+$, 70), 233 ($M+MeOH^+$,100), 218 ($M+H_2O^+$, 20), 201 ($M+H^+$, 4).



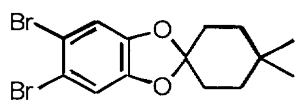
24

To a solution of 3,4-dicyano-1,2-isopropylidenedioxybenzene **22** (0.20 g, 1.00 mmol) in pentanol (4 mL) at 140 °C was added lithium (0.10 g, 14.5 mmol). The reaction was heated at 140 °C for 2 h, cooled and glacial acetic acid (15 mL) added. The solvents were then removed under vacuum. The light green solid was dissolved in DMF (30 mL), treated with zinc acetate dihydrate (0.11 g, 0.50 mmol) and heated at 65 °C for 15 h. The green coloured solution was concentrated and washed with water (10 mL). TLC (10% MeOH (10% NH_3):$CHCl_3$) showed there still to be some starting unmetallated phthalocyanine left. This solid was dissolved in DMF (30 mL) and was treated with zinc acetate dihydrate (0.11 g, 0.50 mmol) and potassium carbonate (0.08 g, 0.55 mmol) (to aid complexation). This mixture was heated at 65 °C for 20 h. The green coloured solution was cooled to 25 °C and concentrated under vacuum. The resulting solid was dissolved in $CHCl_3$ (30 mL) and carefully added to a silica column packed in $CHCl_3$. The eluent was gradually changed to 10% MeOH (10% NH_3):$CHCl_3$ which gave more pure, but not completely pure material. The fractions containing the desired material were concentrated and dissolved in $CHCl_3$ (20 mL). This solution was carefully added to a silica column packed in $CHCl_3$ The eluent was gradually changed to 1.5% MeOH (10% NH_3):$CHCl_3$ and then very slowly changed to 5% MeOH (10% NH_3):$CHCl_3$. Early fractions contained the desired product but were strongly contaminated with impurities. Subsequent fractions contained the pure compound, affording pure phthalocyanine **24** (0.13 g, 60%) as a green solid. ^1H NMR (300 MHz, $CDCl_3$) δ 7.15-7.05 (m, 8H), 1.81-1.73 (m, 24H); MS (APCI) *m/z* (%) 864 (M^+, 4); λ_{max} (nm) 667.



26

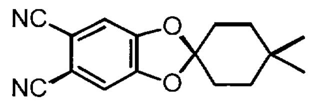
A mixture of 4,4-dimethyl-2-cyclohexenone **25** (5.00 g, 40.26 mmol) and 10% Pd/C (0.10 g) in pentane (70 mL) was hydrogenated at 5 °C at 1 atmosphere of H_2. The reaction was allowed to warm to 25 °C for 18 h. The mixture was filtered through Celite, concentrated to 15 mL and cooled in an i-PrOH-dry ice bath. Filtration afforded 4,4-dimethylcyclohexanone **26** (2.60 g, 51%) as a white fluffy solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.35 t, *J* = 7.0, 4H), 1.67 (t, *J* = 7.0, 4H), 1.10 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 212.6, 39.2, 38.0, 29.9, 27.5; MS (APCI) *m/z* (%) 159 ($M+MeOH^+$, 20), 127 ($M+H^+$, 100).



27

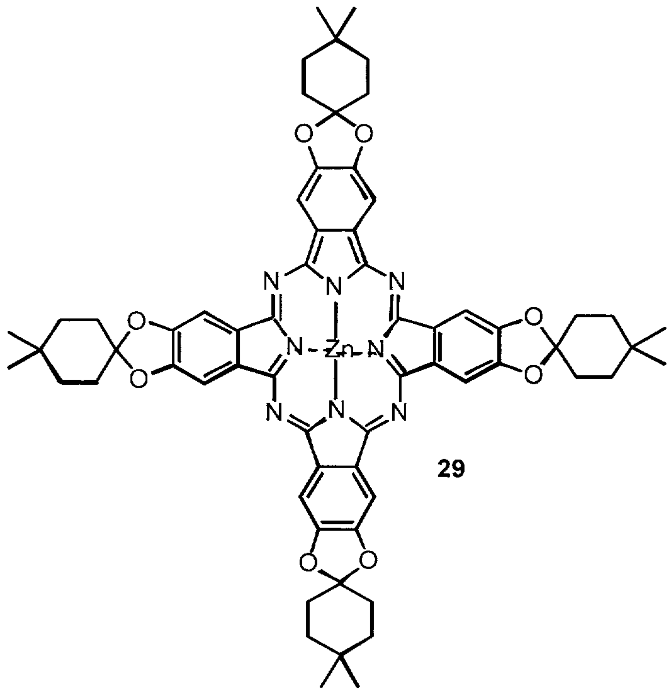
A solution of 3,4-dibromocatechol **19** (2.00 g, 7.46 mmol) and 4,4-dimethylcyclohexanone **26** (0.94 g, 7.46 mmol) in toluene (22 mL) containing p-toluene sulfonic acid mono-hydrate (0.074 g, 0.39 mmol) was set up with a Dean-Stark head, and was heated at 130 °C for 15 h. Once cooled to 25 °C, the solution was washed with aqueous $NaHCO_3$ solution (10 mL), water (10 mL), dried ($MgSO_4$), filtered and concentrated. The mixture was shown by ^{1}H NMR to still contain catechol, and so the mixture was dissolved in diethyl ether (25

mL). This solution was washed with 10% NaOH solution (3 x 20 mL), water (20 mL), dried ($MgSO_4$), filtered and concentrated. The resulting yellow solid was recrystallised from hexanes to give 4,5-dibromo-1,2-di-*O*-(4',4'-dimethylcyclohexylidene)catechol **27** (0.71 g, 25%). Mp 126 -127 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 6.97 (s, 2H), 1.91 t, J = 6.0, 4H), 1.51 (t, J = 6.0, 4H), 0.99 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 147.9, 121.3, 114.5, 113.1, 35.7, 31.4, 29.3, 27.8; MS (EI) m/z (%) 376 (M^+, 100), 314 (50), 304 (50).



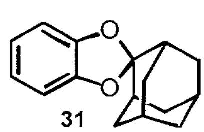
28

A mixture of **27** (0.64 g, 1.70 mmol), CuCN (0.61 g, 6.81 mmol) in DMF (7 mL) was heated to 150 °C for 5h. The mixture was then cooled to 25 °C, treated with ammonia water (25 mL) and stirred for 30 min. The mixture was filtered, washed with water (10 mL) and air dried for 18 h. The resulting solid was extracted with diethyl ether using a Soxlet extractor for 3 days. The solvent was then evaporated to produce 4,5-dicyano-1,2-di-*O*-(4',4'-dimethylcyclohexylidene)catechol **28** (1.58 g, 49%) as a white solid. Mp 159 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.05 (s, 2H), 1.97 (s, 4H), 1.56 (s, 4H), 1.02 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.4, 124.4, 115.8, 112.5, 110.0, 35.5, 31.5, 29.3, 27.7; MS (EI) m/z (%) 268 (M^+, 100), 252 (30), 212 (70), 197 (100).



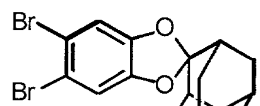
29

To a solution of **28** (90 mg, 0.33 mmol) and anhydrous zinc acetate (30 mg, 0.16 mmol) in pentanol (2 mL) at 140 °C was added lithium (33 mg, 4.75 mmol). This mixture was heated at 140 °C for 17 h. The reaction was cooled to 25 °C, solvent was removed under vacuum and washed with water (10 mL). The resulting solid was dissolved in $CHCl_3$ and purified on a silica column ($CHCl_3$ - 1% MeOH (10% NH_3):$CHCl_3$) to give the desired phthalocyanine **29** (55 mg, 60%) as a blue/green solid. ^1H NMR (300 MHz, $CDCl_3$) δ 8.15 (bs, 8H), 2.35 (bs, 16H), 1.88 (bs, 16H), 1.24 (*s, 26H); MS (FAB) *m/z* (%) 1137 ($M+H^+$, 6), 1136 (M^+, 5)); λ_{max} (nm) 669.



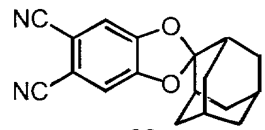
31

A mixture of catechol **18** (4.03 g, 36.6 mmol), 2-adamantanone **30** (5.00 g, 33.3 mmol) and p-toluene sulfonic acid mono-hydrate (0.13 g) in benzene (125 mL) was heated at 80 °C for 22 h. The solution was cooled to 25 °C and washed successively with 10% NaOH solution (3 x 50 mL), water (50 mL), dried ($MgSO_4$), filtered and concentrated. The resulting light yellow solid was recrystallised from benzene to give 1,2-di-*O*-(adamantylidene)catechol **31** (6.77 g, 84%) as an off-white solid. Mp 130 °C (lit.[x] 126 -127 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.75 (s, 4H), 2.18-1.75 (m, 14H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 147.7, 120.8, 108.4, 37.1, 36.7, 34.4, 26.7; MS (FAB) *m/z* (%) 242 (M^+, 95).



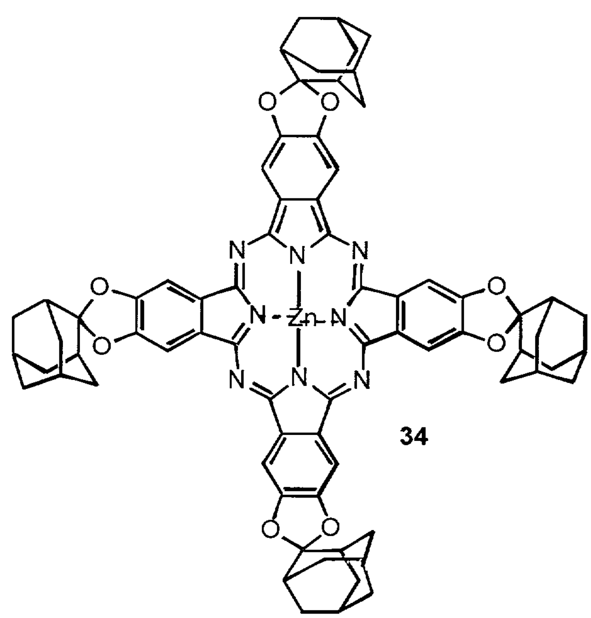
32

A solution of **31** (2.42 g, 10 mmol) in CCl_4 (10 mL) cooled to 0 °C was treated with Br_2 (3.20 g, 1.02 mL, 20 mmol) dissolved in CCl_4 (1 mL). The mixture was stirred at 0 °C for 30 min. The mixture was diluted with $CHCl_3$ (29 mL) and washed with 10% aqueous NaOH solution (2 x 20 mL), water (20 mL), dried ($MgSO_4$), filtered and concentrated. The resulting solid was recrystallised twice from hexanes to give pure 4,5-dibromo-1,2-di-*O*-(adamantylidene)catechol **32** (1.92 g, 48%) as a white solid. TLC R_f 0.75 1:19 Ethyl acetate:hexanes; Mp 195 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.99 (s, 2H), 2.16-1.75 (m, 14H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 148.2, 124.0, 114.3, 113.0, 36.9, 36.8, 34.3, 26.5; MS (FAB) *m/z* (%) 400 (M^+, 25).



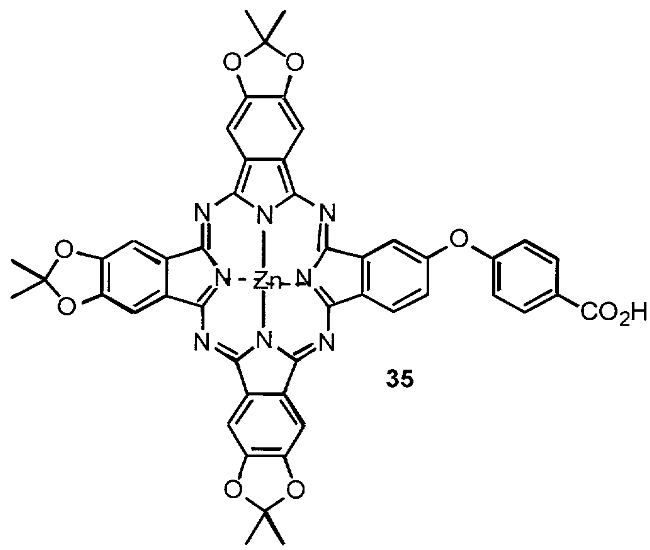
33

A mixture of **32** (1.43 g, 3.57 mmol) and CuCN (1.28 g, 14.30 mmol) in DMF (15 mL) was heated to 150 °C for 3 h. The mixture was then cooled to 25 °C, treated with ammonia water (50 mL) and stirred for 30 min. The mixture was filtered, washed with water (10 mL) and air dried for 18 h. The resulting solid was extracted with dithyl ether using a Soxlet extractor for 3 days. The solvent was then evaporated and the resulting solid was recrystallised from warm benzene to give 4,5-dicyano-1,2-di-*O*-(adamantylidene)catechol **33** (0.48 g, 46%) as a green solid. Mp 210 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.06 (s, 2H), 2.21-1.79 (m, 14H); 13C NMR (75 MHz, CDCl3) δ 151.6, 127.2, 115.8, 112.4, 109.9, 37.0, 36.7, 34.2, 26.3; MS (FAB) m/z (%) 315 (M+Na+, 10), 293 (M+H+, 20).



34

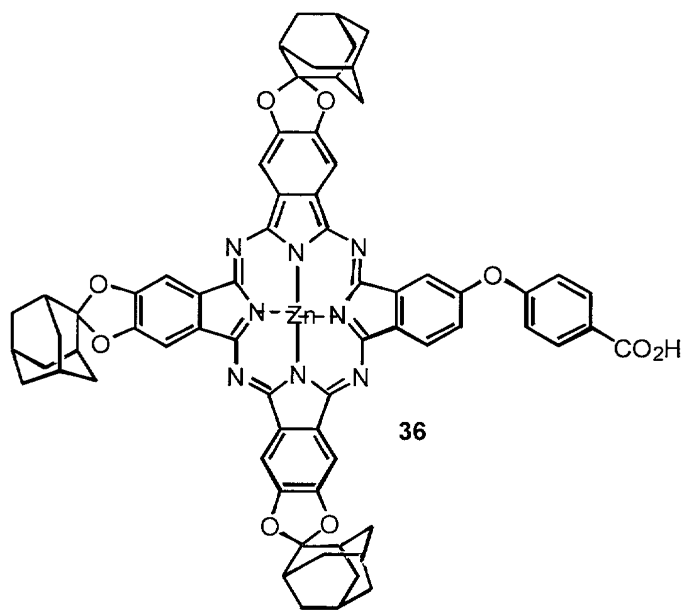
To a solution of phthalonitrile **33** (0.24 g, 0.82 mmol) and anhydrous zinc acetate (75 mg, 0.41 mmol) in pentanol (5 mL) at 140 °C was added lithium (80 mg, 11.5 mmol). The solution was heated at 140 °C for 18 h. The solution was then cooled to 25 °C and TLC analysis (10% MeOH (10% NH3):CHCl3) showed there to be only the desired phthalocyanine **34** present. TLC Rf 0.80 10% MeOH (10% NH3):CHCl3; 1H NMR (300 MHz, CDCl3) δ 8.60 (bs, 8H), 1.61-2.67 (m, 56H); MS (FAB) m/z (%) 1235 (M+H+, 5); λ_{max} (nm) 668.



35

To a solution of 3,4-dicyano-1,2-isopropylidenedioxybenzene **22** (0.21 g, 1.08 mmol), **16** (0.19 g, 0.73 mmol) and anhydrous zinc acetate (0.10 g, 0.55 mmol) in pentanol (10 mL) at 140 °C was added lithium (100 mg, 14 mmol). The reaction was heated at 140 °C for 20 h, cooled to 25 °C and concentrated under vacuum. The resulting solid was dissolved in the minimum volume of DMF and added to a silica column packed using diethyl ether:DMF 98:2. The

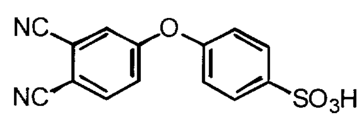
eluent diethyl ether:DMF 98:2 was used until all of the first compound to come off the column had come off. The eluent was then very gradually changed to DMF. At this time the next fraction came off, which contained the desired compound. 3:1 Mixed phthalonitrile **35** (72 mg, 22%) was isolated as a blue/green solid. TLC Rf 0.24 20% MeOH (10% NH3):CHCl3; 1H NMR (300 MHz, DMSO-d6) δ 8.71 (m, 4H), 8.13-8.01 (m, 5H), 7.71-7.23 (m, 4H), 5.76 (s, 1H), 1.96 (s, 18H); MS (FAB) m/z (%) 930 (M+H+, 2) 929 (M+, 2); λ_{max} (nm) 666.



36

To a solution of phthalonitrile **33** (0.12 g, 0.41 mmol), **16** (72 mg, 0.28 mmol) and anhydrous zinc acetate (38 mg, 0.21 mmol) in pentanol (4 mL) at 140 °C was added lithium (40 mg, 5.30 mmol). The reaction was heated at 140 °C for 17 h, the solution was then cooled to 25 °C and the solvent removed under vacuum. The resulting solid was dissolved in the minimum volume of DMF and added to a silica column packed

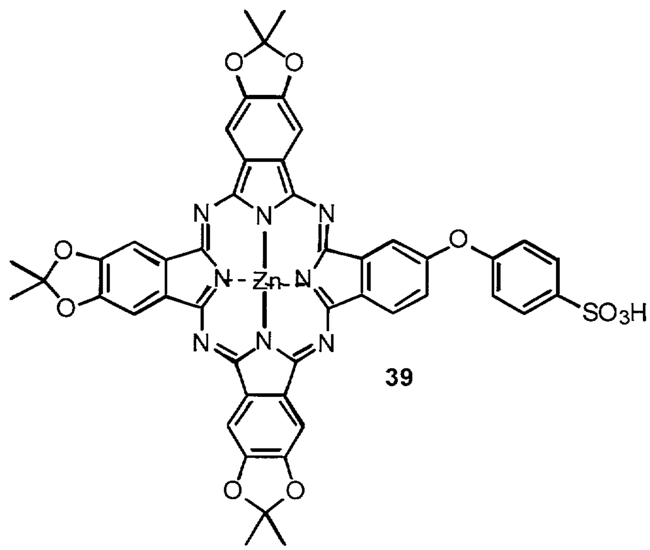
using diethyl ether:DMF 98:2. The eluent diethyl ether:DMF 98:2 was used until all of the first compound to come off the column had come off. The eluent was then very gradually changed to DMF. At this time the next fraction came off, which contained the desired compound which was slightly contaminated. The relevant fractions were concentrated, the resulting solid was dissolved in the minimum volume of DMF and added to a silica column packed using diethyl ether:DMF 98:2. The eluent diethyl ether:DMF 98:2 was used until all of the first compound to come off the column had come off. The eluent was then very gradually changed to DMF. 3:1 Mixed phthalonitrile **36** (68 mg, 41%) was isolated as a blue/green solid. TLC R_f 0.25 20% MeOH (10% NH_3):$CHCl_3$; ^1H NMR (300 MHz, DMSO-d_6) δ 9.38-9.33 (m, 1H), 8.94-8.90 (m, 1H), 8.58-8.42 (m, 4H), 8.16 (d, J = 8.4, 2H), 8.04-7.92 (m, 1H), 7.58 (s, 1H), 7.49 (d, J = 8.7, 2H), 7.27 (s, 1H), 2.33-1.73 (m, 42H); MS (FAB) m/z (%) 1205 (M^+, 0.5); λ_{max} (nm) 667.



38

To a solution of 4-nitrophthalonitrile **11** (1.73 g, 10.00 mmol) in dry DMSO (20 mL) was added 4-hydroxy benzensulfonic acid sodium salt dihydrate (3.98 g, 15.0 mmol), potassium carbonate (2.07 g, 15.00 mmol) and 4 Å molecular sieves. Further potassium carbonate (2.07 g, 15.00 mmol) was added after 4 h. The reaction was stirred for 3 days. The mixture was poured into water (150 mL) and the pH of the solution adjusted to 0 using HCl. The solution was then

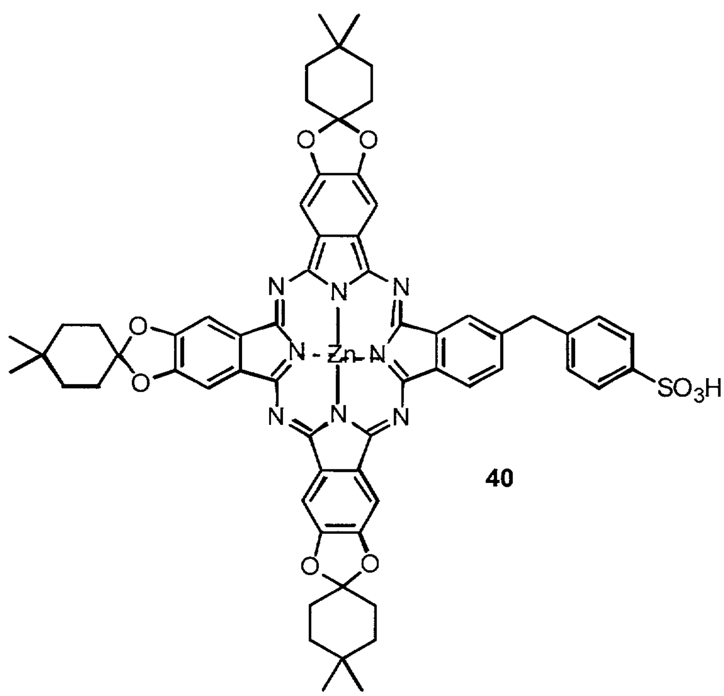
carefully evaporated until a precipitate was seen to form. The resulting solid was filtered and washed with ethanol (50 mL) to give pure 4-(3,4-dicyano-phenoxy)-benzenesulfonic acid **38** (2.10 g, 70%) as a white solid. TLC R_f 0.67 7:7:5 i-PrOH:Ethyl Acetate:Water; ^{1}H NMR (300 MHz, DMSO- d_6) δ 8.11 (d, J = 8.7, 1H), 7.86 (J = 2.5, 1H), 7.72 (ap. dt, J = 8.7, 2.6, 2H), 7.41 (dd, J = 8.7, 2.6, 1H), 7.14 (ap. dt, J = 8.7, 2.6, 2H); MS (FAB) m/z (%) 299 ($M-H^+$, 100).



39

To a solution of 3,4-dicyano-1,2-isopropylidenedioxybenzene **22** (0.105 g, 0.53 mmol), **38** (0.11 g, 0.37 mmol) and anhydrous zinc acetate (50 mg, 0.28 mmol) in pentanol (5 mL) at 140 °C was added lithium (50 mg, 7 mmol). The reaction was heated at 140 °C for 22 h, then cooled to 25 °C and concentrated under vacuum. The resulting solid was dissolved in a mixture of MeOH:DMF:10% ammonium formate buffer (65:25:10), the same solvent mixture was also used to pack a reverse-phase silica column. The reaction mixture was eluted with this system,

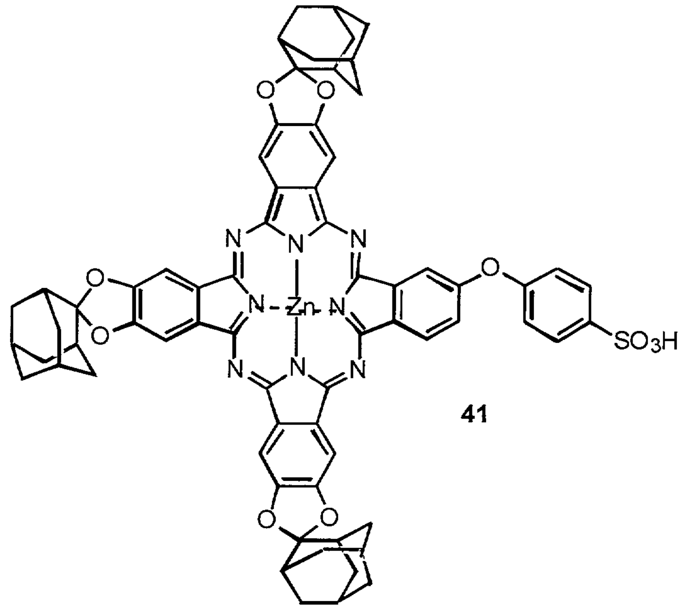
yielding some fractions containing improved purity product. These were concentrated, dissolved in the same MeOH:DMF:10% ammonium formate buffer (65: 25: 10), the same solvent mixture was also used to pack a reverse-phase silica column. The product could now be isolated pure **39** (75 mg, 44%). TLC R_f 0.20 20% MeOH (10% NH_3):$CHCl_3$; ^1H NMR (300 MHz, DMSO-d_6) δ 9.42-9.36 (m, 1H), 8.72-8.50 (m, 4H), 8.41-8.21 (m, 1H), 7.78 (d, J = 8.7, 2H), 7.64-7.53 (m, 1H), 7.32 (d, J = 9.0, 2H), 7.25 (s, 1H), 7.11 (s, 1H), 1.98-1.95 (m, 6H), 1.71-1.65 (m, 12H); MS (FAB) m/z (%) 966 ($M+H^+$, 1.5), 965 (M^+, 1.5); λ_{max} (nm) 665.



40

To a solution of **28** (50 mg, 0.186 mmol), **38** (38 mg, 0.125 mmol) and anhydrous zinc acetate (17 mg, 0.093 mmol) in pentanol (2 mL) at 140 °C was added lithium (17 mg, 2.43 mmol). The reaction was heated at 140 °C for 18 h, cooled to 25 °C and concentrated under vacuum. The resulting solid was dissolved in a

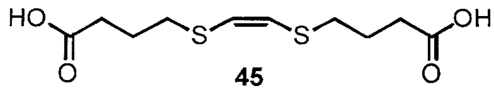
mixture of MeOH: DMF: 10% ammonium formate buffer (60: 20: 20), the same solvent mixture was also used to pack a reverse-phase silica column. The column was eluted with this system, which allowed the more polar compounds to come off whilst leaving the desired compound still on the column. Once the more polar compounds had finished coming off the solvent system was changed to MeOH: DMF: THF (55: 25: 20) and this gave almost pure product. These fractions were concentrated, dissolved in the MeOH:DMF:10% ammonium formate buffer (60: 20: 20), the same solvent mixture was also used to pack a reverse-phase silica column. The column was eluted with this system, which allowed the more polar compounds to come off whilst leaving the desired compound still on the column. Once the more polar compounds had finished coming off, the solvent system was changed to MeOH: DMF: THF (55: 25: 20) and this gave pure phthalocyanine **40** (26 mg, 36%) as a blue/green solid. TLC R_f 0.21 20% MeOH (10% NH_3):$CHCl_3$; ^1H NMR (300 MHz, DMSO- d_6) δ 9.38-9.28 (m, 1H), 8.89-8.82 (m, 1H), 8.67-8.49 (m, 4H), 7.82 (d, J = 7.5, 2H), 7.37 (d, J = 8.1, 2H), 7.25 (d, J = 7.2, 1H), 7.03 (s, 1H), 6.87 (d, J = 7.5, 1H), 2.27 (bs, 4H), 1.96 (bs, 4H), 1.74 (bs, 4H), 1.49 (bs, 4H), 1.22-0.74 (m, 26H); MS (FAB) m/z (%) 1171 (M^+, 30); λ_{max} (nm) 666.



41

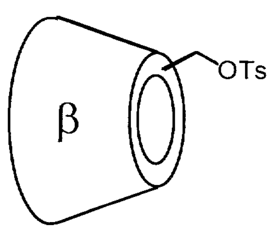
To a solution of **33** (54 mg, 0.186 mmol), **38** (38 mg, 0.125 mmol) and anhydrous zinc acetate (17 mg, 0.093 mmol) in pentanol (2 mL) at 140 °C was added lithium (17 mg, 2.43 mmol). The reaction was heated at 140 °C for 4 h, then cooled to 25 °C and concentrated under vacuum. The resulting solid was dissolved in a mixture of MeOH: DMF: 10% ammonium formate buffer (60: 20: 20), the same solvent mixture was also used to pack a reverse-phase silica column. The column was eluted with this solvent system, which allowed the more polar compounds to come off whilst leaving the desired compound still on the column. Once the more polar compounds had finished coming off, the solvent system was changed to MeOH: DMF: THF (55: 25: 20), and this gave pure phthalocyanine **41** (30 mg, 39%) as a blue/green solid. TLC R_f 0.25 20% MeOH (10% NH_3):$CHCl_3$; ^1H NMR (300 MHz, DMSO- d_6) δ 9.378 (bs, 2H), 8.93-8.85 (m, 1H), 8.70-8.52 (m, 3H), 7.80 (d, J = 7.5, 2H), 7.54 (s, 1H), 7.50 (d, J = 9.9, 1H), 7.37

(d, J = 7.5, 2H), 7.05 (s, 1H), 2.26-1.72 (m, 42H); MS (FAB) m/z (%) 1243 (M^+,10); λ_{max} (nm) 667.



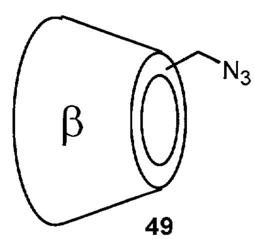
45

4,4'-Dithioldibutyric acid (2.00g, 8.38 mmol) was placed in a three-neck flask and was evacuated and purged with argon three times. Liquid ammonia (100 mL) was condensed into the flask using a cold trap. Sodium metal pieces were added until the blue colour remained. This solution was then stirred for 40 min and sodium pieces were added if the colour faded out. The reaction was quenched with the addition of the minimum amount of ammonium chloride, then *cis*-1,2-dichloroethylene (0.82 g, 8.38 mmol) was added and the reaction was stirred for 4 h. The ammonia was allowed to evaporate off, then the residue was dissolved in water (50 mL) and the solution neutralised using dilute HCl. The mixture was then filtered and dried under vacuum which gave pure 4-[2-(3-carboxy-propylsulfonyl)-vinylsulfanyl]-butyric acid **45** (1.99 g, 90%) as a white solid. Mp 120 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.11 (2, 2H), 6.19 (s, 2H), 2.71 (t, J = 7.2, 4H), 2.30 (t, J = 7.2, 4H), 1.75 (t, J = 7.2, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.0, 123.0, 32.3, 32.1, 25.5.



48

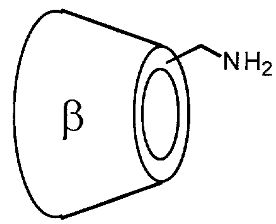
β-Cyclodextrin **47** (40.0 g, 35.3 mmol) was dissolved in hot water (900 mL) (80 °C), then cooled down to 25 °C while being vigorously stirred. p-Toluenesulfonyl imidazole (15.60 g, 70.2 mmol) was added as finely grounded powder. The suspension was stirred for 2 h. Sodium hydroxide (18 g) was dissolved in water (50 mL) and the solution was added to the reaction mixture over a period of 20 minutes. After stirring for another 10 minutes, the mixture was filtered through a frit funnel. The reaction was quenched with ammonium chloride (48.2 g), and the solution was concentrated to about half of its volume. After cooling at 0 °C for an hour, the precipitate was filtered and washed with water (50 mL), acetone (50 mL), then lyophilized to afford the desired product **48** (25 g, 52%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.24(d, 2H), 7.43(d, 2H), 5.60-5.83(m, 14H), 4.74-4.90(m, 7H), 4.30-4.53(m, 6H), 4.29(dd, 1H), 3.40-3.73(m, 28H), 2.41(s, 3H).



49

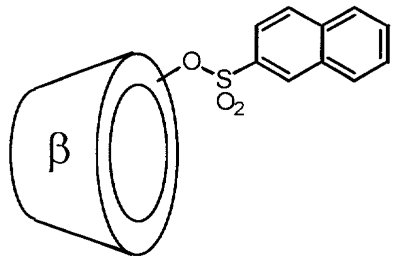
6-Monotosyl-β-cyclodextrin **48** (2.00 g, 1.57 mmol) and sodium azide (2.04 g, 31.4 mmol) were dissolved in DMF (20 mL) and heated to 80 °C for 10 h. The reaction was then cooled to 25 °C and poured into acetone (1 L). The resultant precipitate was

filtered and purified by reverse-phase column using a H_2O/MeOH solvent gradient (MeOH :H_2O 0-80%). This gave pure 6-mono-azido-β-cyclodextrin **49** (1.60 g, 91%) as a white solid. ^1H NMR (300 MHz, DMSO-d_6) 5.80-5.62 (m, 13H), 5.60 (d, 1H), 4.87 (d, 1H), 4.85-4.78 (m, 6H), 4.55-4.40 (m, 6H), 3.80-3.46 (m, 28H), 3.46-3.20 (m, overlap with water peak).



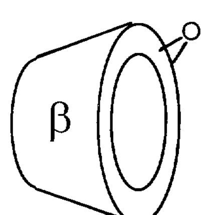
50

To a solution of 6-mono-azido-β-cyclodextrin **49** (1.00 g, 0.86 mmol) in DMF (20 mL) was added triphenylphosphine (0.50 g, 1.9 mmol) and the reaction was stirred at 90 °C for 18 h. The reaction mixture was then cooled to 25 °C and poured into acetone (1 L). The precipitate was then filtered, washed with acetone (100 mL) and dissolved in water (10 mL). After lyophilisation, the desired 6-monoamino-β-cyclodextrin **50** (0.85 g, 86%) was isolated as a white solid. ^1H NMR (300 MHz, DMSO-d_6) δ 5.90-5.55 (m, 14H), 4.89 (d, 1H), 4.88-4.75 (m, 6H), 4.60-4.35 (m, 6H), 3.75-3.49 (m, 26H), 3.03 (m, 1H), 2.80 (m, 1H); MS (FAB) *m/z* (%) 1134 (M+H$^+$, 10).



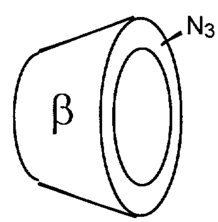
51

β-Cyclodextrin **47** (20.0 g, 7.6 mmol) was dissolved in a mixture of water (150 mL) and MeCN (50 mL) and its pH was adjusted to 12.0 using 4.0 M NaOH aqueous solution. The solution was heated to 40 ^{o}C and was stirred vigorously. 2-Naphthalenesulphonyl chloride (10 g, 44.1 mmol) was added, and the mixture was stirred for another two minutes until its pH dropped to 7. The mixture was then filtered, and the filtrate was diluted with water (1 L) and loaded onto a reverse phase silica column. After elution with water-methanol liner gradient (0 - 80% MeOH/H_2O), 3-mononapthalenesulfonyl-β-cyclodextrin **51** (3.20 g, 16%) was obtained as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.68(s, 1H), 8.21 (d, 1H), 8.14 (d, 1H), 8.06 (d, 1H), 7.98 (d, 2H), 7.79-7.67 (m, 2H), 6.02 (d, 1H), 5.88-5.60 (m, 10H), 5.51 (d, 1H), 4.97-4.75 (m, 7H), 4.68 (t, 1H), 4.60-4.48 (m, 5H), 4.34 (t, 1H), 4.16 (d, 1H), 3.88-3.42 (m, 28H), 3.42-3.15 (m, overlap with water peak).



52

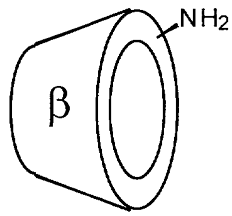
2-Mononaphthalenesulfonyl-β-cyclodextrin **51** (3.20 g, 2.43 mmol) was dissolved in 10% Na_2CO_3 aqueous solution (50 mL) and was stirred at 50 ^{O}C for 10 hours. The mixture was loaded on a reverse phase silica column and was eluted with water-methanol gradient (0 - 80% $MeOH/H_2O$). The fractions containing the desired product was collected and methanol was removed under reduced pressure to give β-cyclodextrin monoalloepoxide **52** (3.00 g, 96%) as a white solid. ^1H NMR (400 MHz, DMSO- d_6) δ 5.90-5.45 (m, 9H, 5.33 (bs, 1H), 5.23 (d, 1H), 5.19 (d, 1H), 5.07 (bs, 1H), 4.88-4.73 (m, 6H), 4.60 (t, 1H), 4.56-4.32 (m, 6H), 3.90 (d, 1H), 3.82-3.41 (m, 28H), 3.41-3.13 (m, overlap with water peak).



53

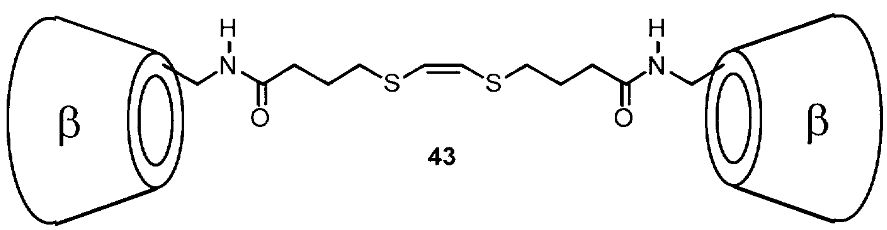
β-Cyclodextrin monoalloepoxide **52** (2.00 g, 1.79 mmol) and sodium azide (0.50 g, 7.69 mmol) were dissolved dry DMF (20 mL) and heated to 90 ^{O}C for 18 h. The DMF was removed under vacuum and the residue was dissolved in water, loaded on a reverse phase silica column, and eluted slowly and very carefully with water-methanol linear gradient (methanol 20-80%, v/v). Two fractions (about 1:3) that contained cyclodextrin were collected and the methanol was removed under reduced pressure. The major fraction contained the desired 2-monoazido-β-cyclodextrin **53** (1.20 g, 60%) as a white solid. ^1H NMR (400 MHz, DMSO- d_6) δ 5.90-5.50 (m, 11H), 4.95-4.77 (m, 7H),

4.67 (d, 1H), 4.62-4.42 (m, 7H), 3.83 (bs, 1H), 3.80-3.65 (m, 28H), 3.65-3.30 (m, overlap with water peak).



54

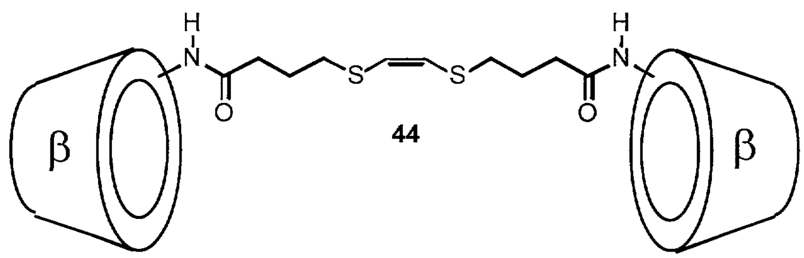
2-Monoazido-β-cyclodextrin **53** (1.00 g, 0.86 mmol) and triphenylphosphine (0.50 g, 1.90 mmol) were dissolved in DMF (20 mL). The reaction mixture was stirred at 90 °C for 18 h, then poured into acetone (1 L). After filtration, the solid was washed with acetone (50 mL) and dissolved in the minimum volume of water. 2-Amino-β-cyclodextrin **54** (0.92 g, 93%) was obtained as a white solid after lyophilisation. ^{1}H NMR (400 MHz, D_2O) δ 5.00-4.91 (m, 7H), 3.91-3.68 (m, 28H), 3.60-3.42 (m, 28H), 3.26 (t, 1H).



43

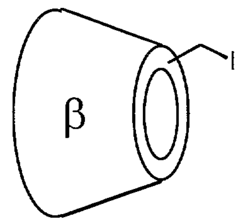
To a solution of 2-amino β-cyclodextrin **54** (0.50 g, 0.44 mmol) in DMF (20 mL) was added **45** (60 mg, 0.22 mmol), 1-hydroxybenzotriazole (HOBT) (89 mg, 0.66 mmol) and 1,3-dicyclohexylcabodiimide (DCC) (0.14 g, 0.66 mmol). The mixture was then heated at 60 °C for 18 h and then poured into acetone (1 L). The

resultant precipitate was filtered and purified by reverse-phase column using a H_2O/MeOH solvent gradient (MeOH 20-80%). This gave pure dimer **43** (0.23 g, 42%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.68 (bs, 2H), 6.16 (s, 2H), 5.80-5.67 (m, 28H), 4.81 (m, 14H), 4.47 (m, 14H), 3.62-3.32 (m, 84H), 2.72-2.66 (m, 4H), 2.21-2.10 (m, 4H), 1.84-1.66 (m, 4H); MS (MALDI) m/z (%) 2517 (M+Na$^+$, 5).



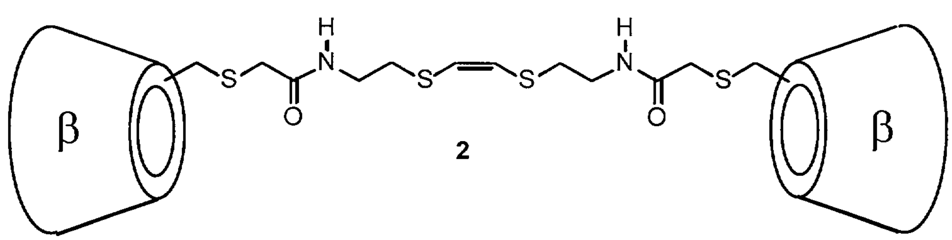
44

To a solution of 6-amino β-cyclodextrin **50** (0.50 g, 0.44 mmol) in DMF (20 mL) was added **45** (60 mg, 0.22 mmol), 1-hydroxybenzotriazole (HOBT) (89 mg, 0.66 mmol) and 1,3-dicyclohexylcabodiimide (DCC) (0.14 g, 0.66 mmol). The mixture was then heated at 60 °C for 18 h and then poured into acetone (1 L). The resultant precipitate was filtered and purified by reverse-phase column using a H_2O/MeOH solvent gradient (MeOH 20-80%). This yielded pure dimer **44** (0.22 g, 40%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.90 (bs, 2H), 6.20 (s, 2H), 5.99 (d, J = 6.1, 2H), 5.79-5.61 (m, 26H), 4.82-4.75 (m, 14H), 4.50-4.45 (m, 14H), 3.62-3.32 (m, 84H), 2.73 (m, 4H), 2.26-2.23 (m, 4H), 1.82-1.79 (m, 4H); MS (MALDI) m/z (%) 2516 (M+Na$^+$, 15).



55

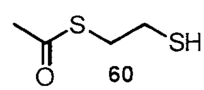
A solution of 6-monotoluenesulfonyl-β-cyclodextrin **48** (20.00 g, 16.8 mmol) and KI (2.00 g, 12.1 mmol) were dissolved in DMF (100 mL). The reaction mixture was stirred under argon at 50 °C for 18 h, and then poured into acetone (1 L). The product was filtered out by frit funnel and washed with acetone, then dissolved in water and lyophilized to give 6-monoiodo β-cyclodextrin **55** as product (17.05 g, 90%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.83-5.60 (m, 14H), 4.90-4.78 (m, 7H), 4.57-4.40 (m, 6H), 3.94-3.54 (m, 28H).



2

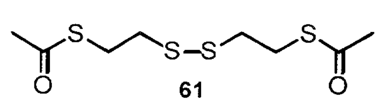
To a solution of diacetate (55 mg, 0.13 mmol) in MeOH (2.5 mL) was added a 25% weight solution of NaOMe in MeOH (0.063 g, 0.29 mmol) and this solution was stirred at 25 °C for 1.25 h. To this solution was then added a solution of 6-monoiodo β-cyclodextrin **55** (0.26 g, 0.21 mmol) in DMF (7 mL) and this solution was heated at 50 °C for 20 h. Upon addition of the cyclodextrin solution the reaction mixture was seen to turn cloudy. This cloudiness had disappeared

after 20 h of heating at 50 °C. This solution was cooled to 25 °C and concentrated. The resulting solid was purified by reverse-phase column using a H_2O/MeOH solvent gradient (MeOH 0-80%). This gave some pure dimer along with some fractions containing impure material. The relevant impure fractions were collected, concentrated and were further purified by reverse-phase column using a H_2O/MeOH solvent gradient (MeOH 0-60%). Overall, this afforded the desired dimer **2** (94 mg, 35%) as a white solid. TLC R_f 0.05 7:7:5 i-PrOH:Ethyl Acetate:Water; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.12-.8.08 (m, 2H), 6.22 (s, 2H), 5.80-5.68 (m, 26H), 4.82 (m, 14H), 4.57-4.46 (m, 12H), 3.77-3.33 (m, 84H), 2.85-2.62 (m, 12H); MS (MALDI) *m/z* (%) 2517 (M+Na$^+$, 5).



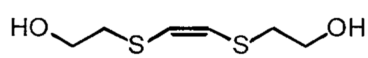
60

A solution of ethanedithiol **59** (3.50 mL, 41.75 mmol) and acetic anhydride (4.00 mL, 41.75 mmol) in pyridine (10 mL) and CH_2Cl_2 (10 mL) was stirred at 25 °C for 18 h. The solvents were removed under vacuum and the resulting oil distilled under high-vac to yield pure thioacetic acid *S*-[2-(2-acetylsulfanyl-ethyldisulfanyl)-ethyl] ester **60** (2.20 g, 39%) as a clear liquid. TLC R_f 0.42 1:9 Ethyl Acetate:Hexanes; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.13-3.06 (m, 2H), 2.75-2.67 (m, 2H), 2.36 (s, 3H), 1.62 (t, *J* = 8.5, 1H).



61

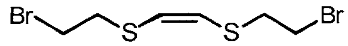
To a solution of mono-acetate **60** (1.03 g, 7.56 mmol) in CH_2Cl_2 (8 mL) and 10% K_2CO_3 in water (8 mL) was slowly added bromine (0.61 g, 0.20 mL, 3.78 mmol). After complete addition the organic layer was separated and the aqueous layer was washed with CH_2Cl_2 (2 x 15 mL). The combined organic layers were dried ($MgSO_4$) and concentrated to give thioacetic acid *S*-[2-(2-acetylsulfanyl-ethyldisulfanyl)-ethyl] ester **61** (0.90 g, 88%) as a yellow oil. TLC R_f 0.20 1:9 Ethyl Acetate:Hexanes; ^1H NMR (300 MHz, $CDCl_3$) δ 3.24-3.19 (m, 4H), 2.90-2.84 (m, 4H), 2.36 (s, 6H); MS (FAB) *m/z* (%) 271 ($M+H^+$, 30), 270 ($M+H^+$, 20).



63

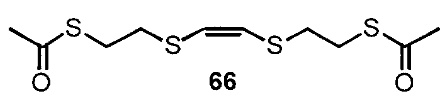
A solution of 2-mercaptoethanol **64** (11.14 g, 10.00 mL, 0.14 mol) and NaOH (5.79 g, 0.145 mol) in EtOH (40 mL) was stirred at 0 °C for 30 min. To this solution was added dropwise a solution of *cis*-1,2-dichloroethylene (6.91 g, 5.48 mL, 0.07 mol) in EtOH (5 mL). This solution was heated at 80 °C for 18 h. This mixture was cooled to 25 °C, diluted with water (100 mL) and washed with diethyl ether (3 x 50 mL). The combined organic layers were washed with water (2 x 75 mL), dried ($MgSO_4$) and concentrated to give the crude product. This was purified by column chromatography on silica (75% ethyl acetate/hexanes - ethyl acetate) to give pure 2-[2-(2-hydroxy-ethylzulfanyl)-vinylsulfanyl] ethanol **63** (9.10 g, 72%) as a light yellow liquid. IR (ν) 3334, 2920, 2866, 1544, 1409, 1283, 1046, 1011, 841, 638; ^1H NMR (300 MHz, $CDCl_3$) δ 6.17 (s, 2H), 3.78 (ap. q, *J* = 6.0, 4H), 2.91 (t, *J* = 5.4, 4H), 2.34 (bt, *J* = 5.4, 2H);

^{13}C NMR (75 MHz, $CDCl_3$) δ 124.9, 61.4, 37.4; MS (FAB) *m/z* (%) 180 (M^+, 100).



65

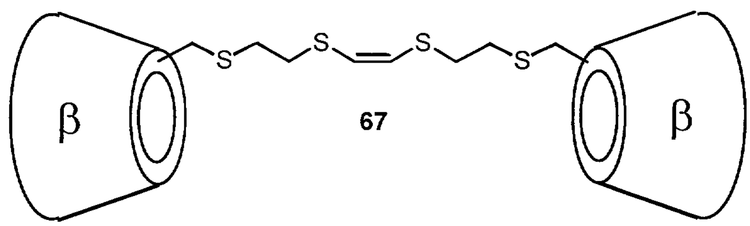
To a 0 °C solution of triphenylphosphine (6.55 g, 24.96 mmol) in CH_2Cl_2 (15 mL) was carefully added bromine (2.93 g, 0.94 mL, 18.3 mmol). This was warmed to 25 °C and diluted with CH_2Cl_2 (60 mL). To this cloudy solution was added a solution of **63** (1.50 g, 8.32 mmol) in CH_2Cl_2 (8 mL), during which time the solution became clear. This mixture was stirred at 25 °C for 2 h. The mixture was then concentrated to give a white solid. This solid was washed with hexanes (20 mL) and diethyl ether (3 x 20 mL). These were combined and concentrated to give 1,2-bis(2-bromo-ethylsulfanyl)-ethene **65** as an ~1:1 mixture with triphenyl phosphine oxide (yield of dibromide = 2.40 g, 95%). IR (ν) 3053, 3010, 1589, 1546, 1476, 1432, 1195, 1120, 747, 721, 664, 619; ^1H NMR (300 MHz, $CDCl_3$) δ 6.14 (s, 2H), 3.47 (t, *J* = 7.8, 4H), 3.10 (t, *J* = 7.8, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 124.4, 35.9, 30.2; MS (FAB) *m/z* (%) 305 ($M+H^+$, 10).



66

To a solution of dibromide **65** /triphenyl phosphine oxide ~1:1 mixture (1.20 g of dibromide, 3.92 mmol) in DMF (15 mL) was added potassium thioacetate (2.24 g, 19.6 mmol) and this mixture was heated to 80 °C for 24 h. The resulting dark mixture was then cooled to 25 °C poured into water (150 mL) and extracted with

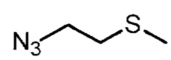
diethyl ether (4 x 50 mL). The combined organic layers were dried ($MgSO_4$) and concentrated to give the crude product. This was purified by column chromatography on silica (15% ethyl acetate/hexanes) to give pure thioacetic acid *S*-{2-[2-(2-acetylsulfanyl-ethylsulfanyl0-vinylsulfanyl]ethyl} ester **66** (0.54 g, 47%) as an off white solid. TLC R_f 0.28 15:85 Ethyl Acetate:Hexanes; Mp 58°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 65.24 (s, 2H), 3.12-3.07 (M, 4H), 2.90-2.84 (m, 4H), 2.35 (s, 6H)); ^{13}C NMR (75 MHz, $CDCl_3$) δ 195.3, 124.2, 33.8, 30.7, 29.9; MS (APCI) *m/z* (%) 295 ($M-H^+$, 100), 270 ($M+H^+$).



67

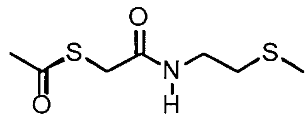
To a solution of diacetate **66** (0.15 g, 0.51 mmol) in MeOH (9 mL) was added 25% weight solution of NaOMe in MeOH (0.24 g, 0.26 mL, 1.11 mmol) and this solution was stirred at 25 °C for 2 h. To this solution was added a solution of 6-monoiodo β-cyclodextrin **55** (1.00 g, 0.80 mmol) in DMF (30 mL) and this solution was heated at 50 °C for 20 h. Upon addition of the cyclodextrin solution the reaction mixture was seen to turn cloudy. This cloudiness had disappeared after 20 h of heating at 50 °C. This solution was cooled to 25 °C and concentrated under vacuum. The resulting solid was purified by reverse-phase column using a H_2O/MeOH solvent gradient (MeOH 0-80%). This gave some pure dimer along with some fractions containing impure material. The relevant impure fractions were collected, concentrated and were

further purified by reverse-phase column using a H_2O/MeOH solvent gradient (MeOH 0-60%). The mixed fractions appeared to be contaminated with of 6-monoiodo β-cyclodextrin **55** and in an attempt to remove this the concentrated mixed fractions were dissolved in 10% NaOH aqueous solution (10 mL) and heated to 60 °C for 3 days. The solution was then cooled to 25 °C, pH adjusted to 7 using dilute HCl and filtered. The resulting solution was purified by reverse-phase column using a H_2O/MeOH solvent gradient (MeOH 0-50%). Overall, this afforded the desired dimer **67** (0.35 g, 36%) as a white solid. TLC R_f 0.13 7:7:5 i-PrOH:Ethyl Acetate:Water; ^1H NMR (300 MHz, DMSO- d_6) δ 6.24 (s, 2H), 5.78-5.67 (m, 28H), 4.84 (s, 14H), 4.49-4.43 (m, 12H), 3.87-3.30 (m, 80H), 3.05-2.64 (m, 12H).



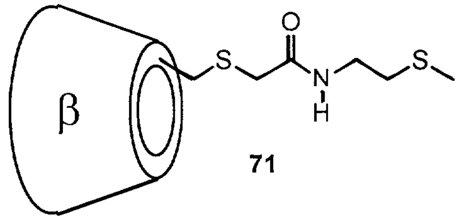
69

To a solution of NaN_3 (3.51 g; 54.0 mmol; 3.0 eq.) in H_2O (20 mL) was added a solution of 2-chloroethyl methyl sulfide **68** (2.0 g; 18.0 mmol; 1.0 eq.) in CH_2Cl_2 (20 mL) and a catalytic amount (~10 mol%) of tetrabutylammonium chloride. This mixture was rigorously stirred at 30 °C for 3 h. The organic layer was separated from the aqueous layer and was dried ($MgSO_4$), filtered and concentrated to yield 1-azido-2-methylsulfanyl-ethane **69** (1.70 g, 81%) as a clear oil. ^1H NMR (300 MHz, $CDCl_3$) δ 3.48 (t, J = 7.0, 2H), 2.70 (t, J = 7.0, 2H), 2.17 (s, 3H).



70

To a solution of 1-azido-2-methylsulfanyl-ethane **69** (1.00 g; 8.6 mmol; 1.0 eq.) and triphenylphosphine (2.24 g; 8.6 mmol; 1.0 eq.) in THF (30 mL), was added water (0.15 mL; 8.6 mmol; 1.0 eq.). The reaction mixture was heated to 35 °C for 3 h, after which it was cooled to 25 °C and then to 0 °C. To this mixture was added freshly distilled triethylamine (1.8 mL; 12.9 mmol; 1.5 eq.). Chloroacetyl chloride (1.46 g; 12.9 mmol; 1.5 eq.) was then added dropwise, and the resulting reaction mixture was allowed to warm to 25 °C. Potassium thioacetate (4.90 g; 43 mmol; 5.0 eq.) was then added to the reaction mixture, which was then heated to 50 °C for 18 h. The solution was then concentrated under reduced pressure, and the residue was purified by column chromatography on silica (gradient: 100% CH_2Cl_2 to 10% MeOH/CH_2Cl_2) to give thioacetic acid *S*-[(methylsulfanyl-ethyl carbamoyl)-methyl]ester **70** (1.20 g, 67%) as an off-white solid. Rf = 0.85 (10% MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.68 (bs, 1H), 3.56 (s, 2H), 3.44 (m, 2H), 2.62 (t, *J* = 6.5, 2H), 2.41 (s, 3H), 2.10 (s, 3H).



71

To a solution of **70** (0.50 g; 2.41 mmol; 30.0 eq.) in MeOH (50 mL) was added NaOH (0.20 g; 5.0 mmol; 62.2 eq.). The resulting mixture was stirred at 50 °C for 10 minutes. TLC showed the disappearance of the starting thioacetate and an appearance of a new spot at Rf = 0.65 (10% MeOH/CH_2Cl_2). The solution was concentrated under reduced pressure. To this residue was added a mixture of β-CD-6-I (0.10 g; 0.08 mmol; 1.0 eq.) and K_2CO_3 (55 mg; 0.4 mmol; 5.0 e.q.) in DMF (20 mL). The reaction flask was evacuated and backfilled with argon three times. The mixture was heated to 55 °C for 24 h. Water (180 mL) was then added to the reaction mixture. This mixture was filtered and was then purified by reverse phase column chromatography eluted with MeOH/H_2O mixture (linear gradient 80% H_2O - 80% MeOH). The methanol of the fractions that contained the product was removed under reduced pressure, and the residual aqueous solution was lyophilized. This gave monomer **71** (80 mg, 78%) as a white solid. Rf = 0.56 (7:7:7:4 iPrOH: EtOAc: H_2O: NH_4OH); ^1H NMR (300 MHz, D_2O) δ 5.05-4.90 (m, 7H), 4.10-3.17 (m, 44H), 2.85 (m, 2H), 2.59 (t, J = 6.6, 2H), 2.14 (s, 3H).

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